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(54) **METHODS, COMPOSITIONS, AND KITS FOR PREDICTING THE EFFECT OF COMPOUNDS ON HOT FLASH SYMPTOMS**

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(57) **ABSTRACT**

The present invention provides methods, compositions, and kits, for determining the effects of one or more candidate compounds on hot flash symptoms. In certain aspects, the methods, compositions, and kits can be used to identify compounds that decrease the incidence of hot flash symptoms. In other aspects, the methods, compositions, and kits can be used to determine whether candidate compounds increase the incidence of undesirable hot flash symptoms when administered to a subject.

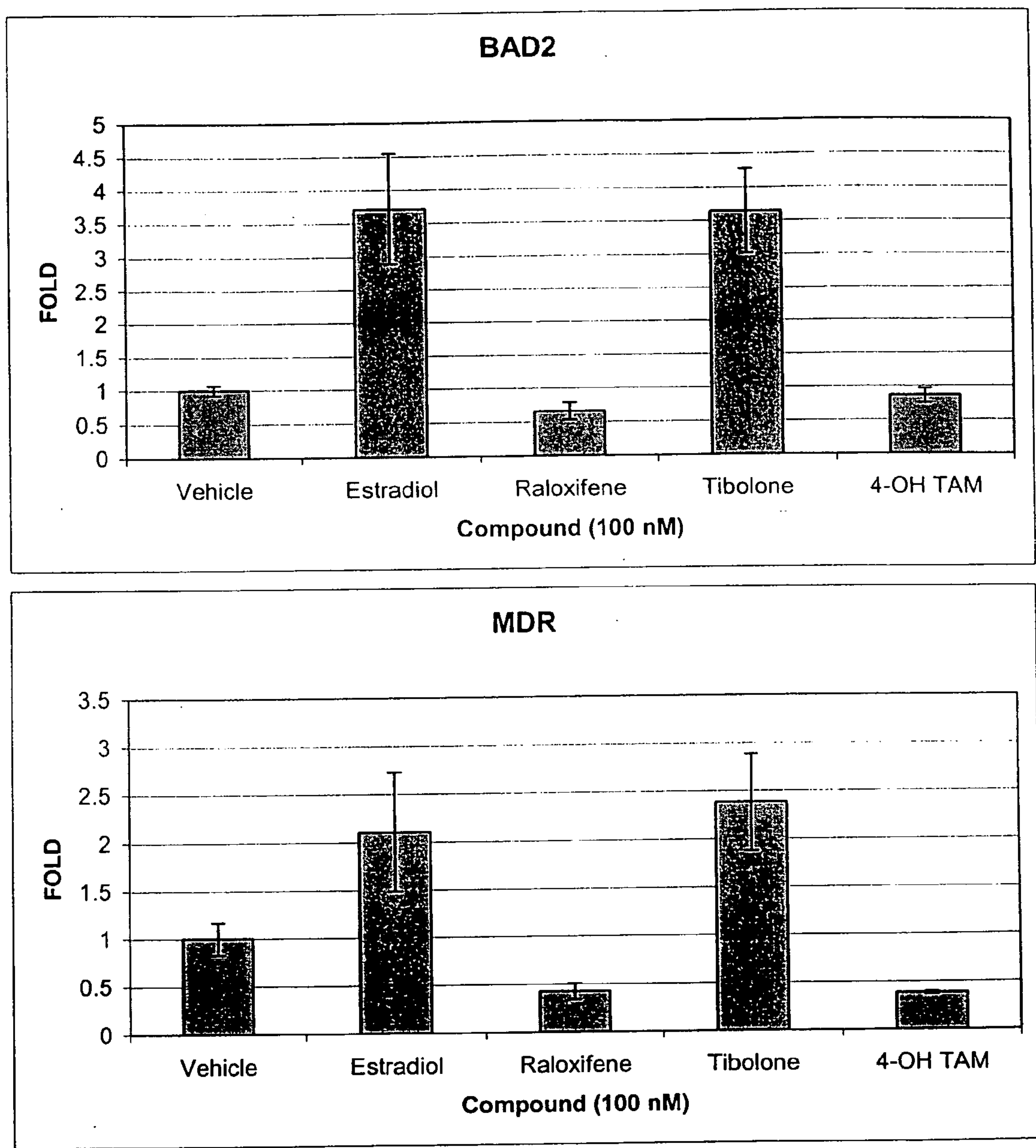


FIG. 1A

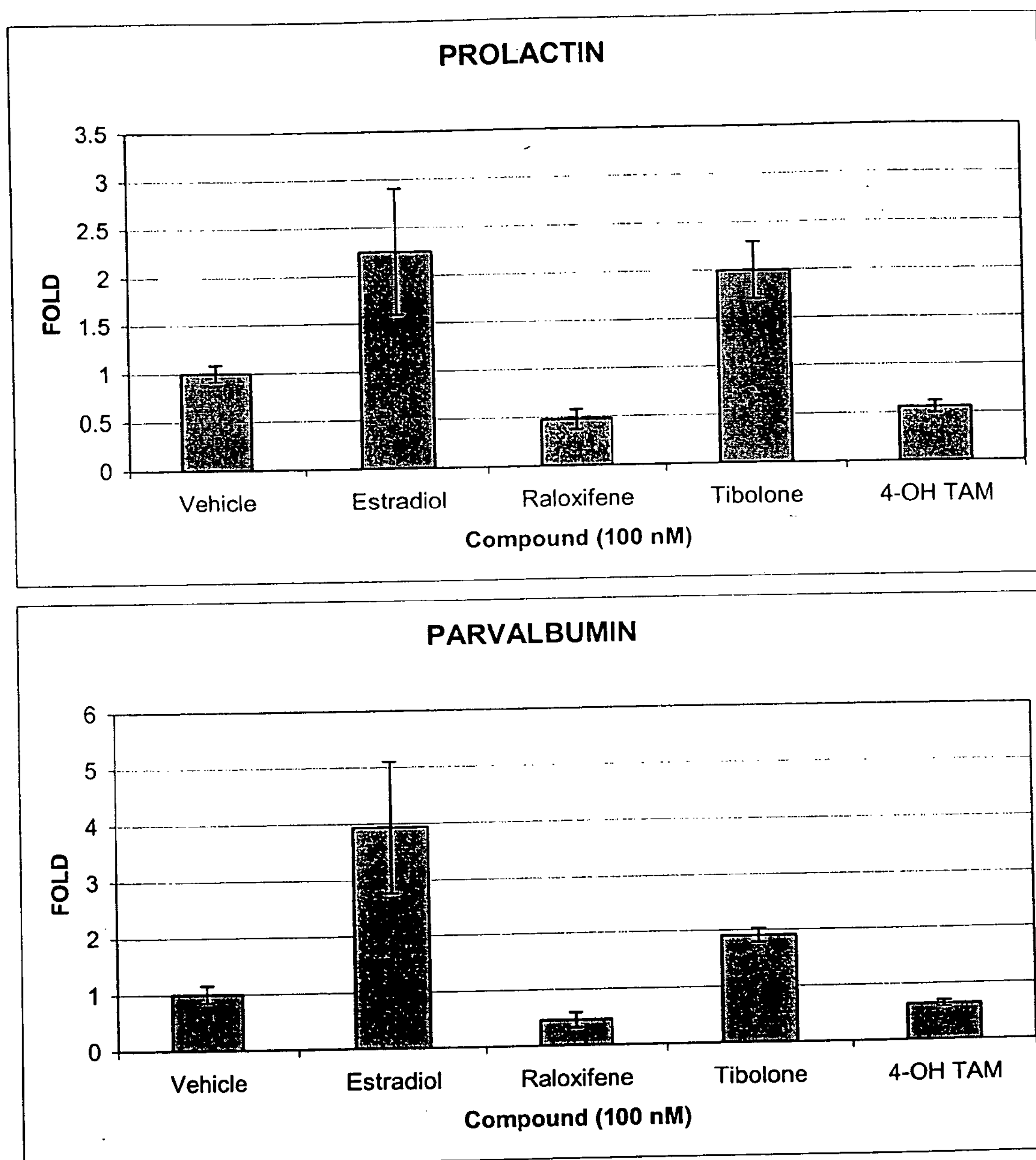


FIG. 1B

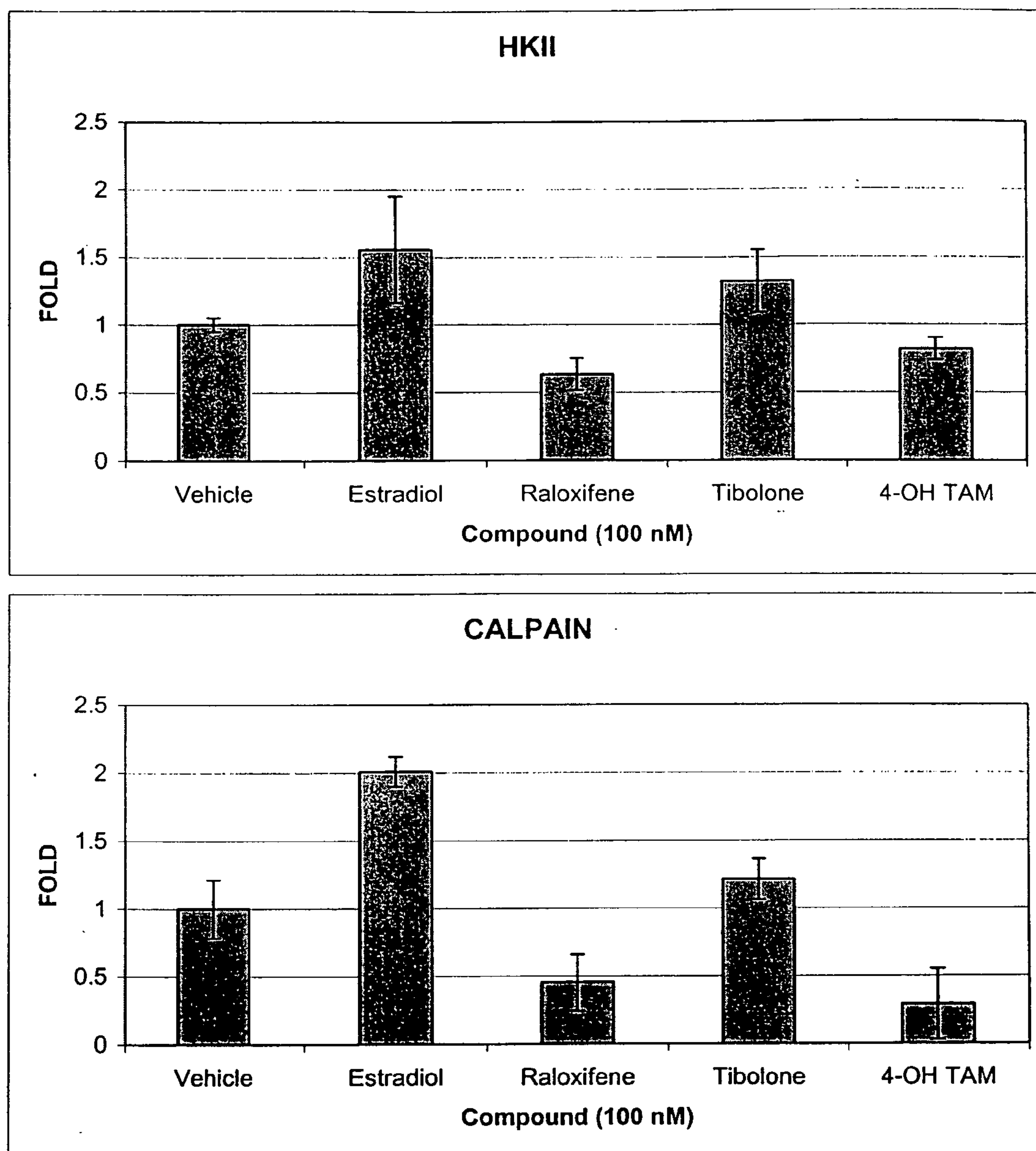
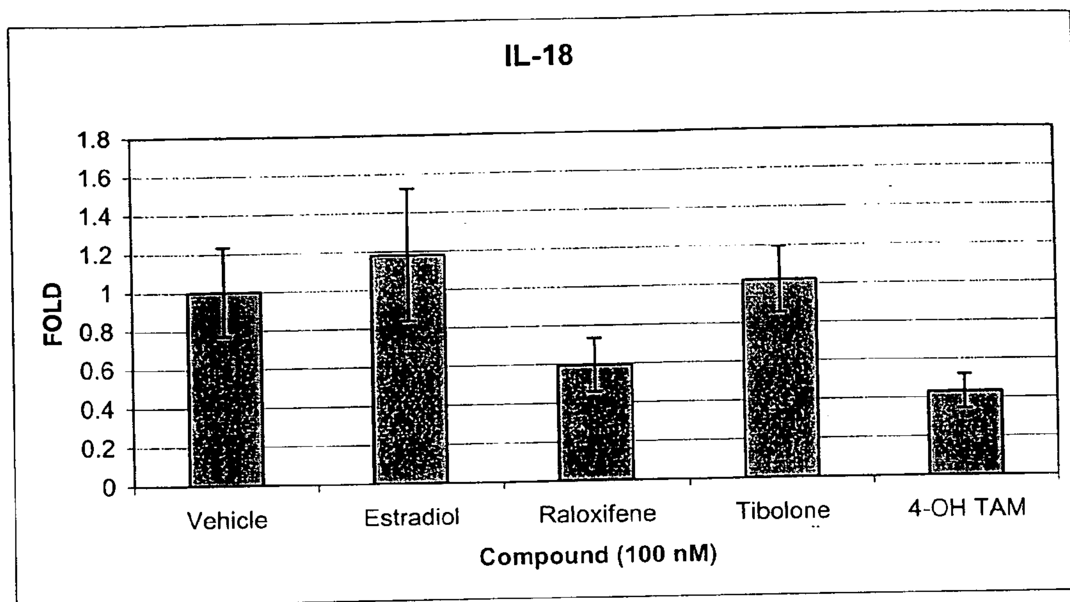


FIG. 1C



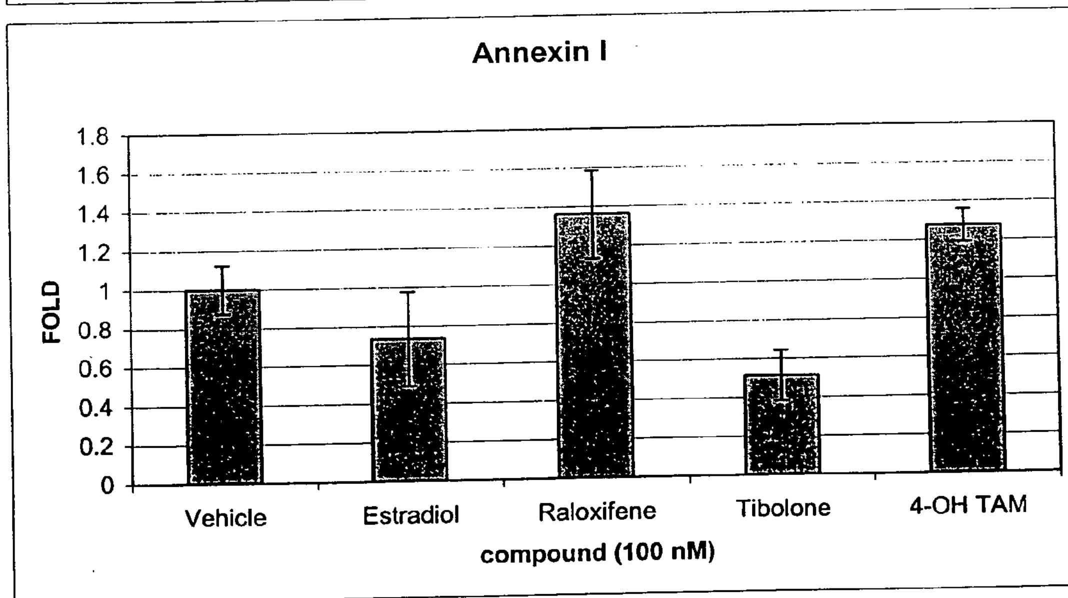


FIG. 1D

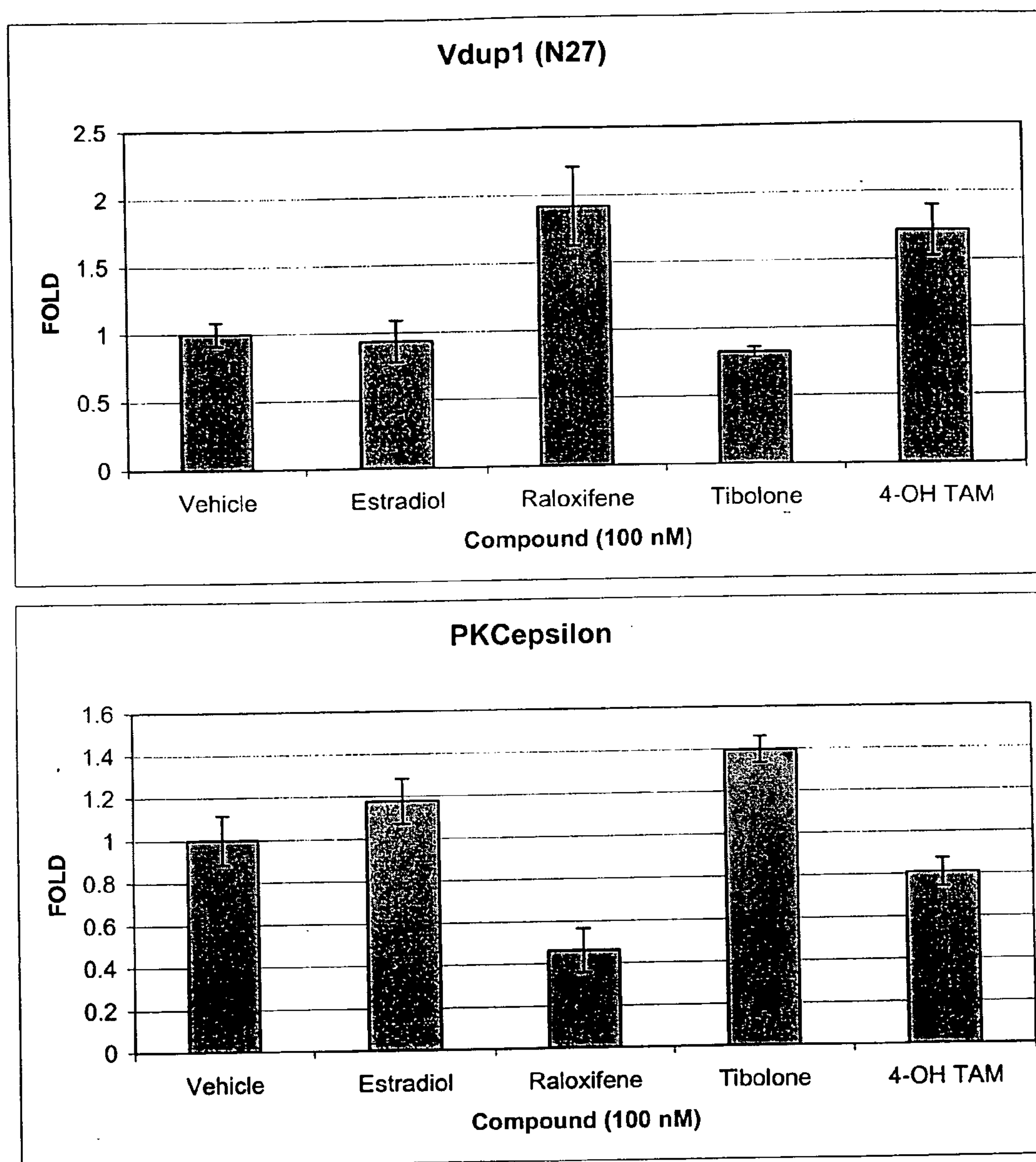


FIG. 1E

METHODS, COMPOSITIONS, AND KITS FOR PREDICTING THE EFFECT OF COMPOUNDS ON HOT FLASH SYMPTOMS

1. REFERENCE TO RELATED APPLICATIONS

[0001] The present application is entitled to and claims the benefit of U.S. Provisional Application No. 60/479,570, filed Jun. 17, 2003, which application is hereby incorporated by reference in its entirety.

2. FIELD OF THE INVENTION

[0002] The field of the invention generally relates to the identification of the effect of candidate compounds on hot flash symptoms based upon their effect on the regulation of expression of genes associated with hot flash symptoms. More specifically, the invention relates to identification of compounds that decrease hot flash symptoms and/or determining the effects of candidate compounds on hot flash symptoms. In another aspect, the invention provides methods of determining whether candidate compounds increase the incidence of hot flash symptoms as an undesirable side effect.

3. BACKGROUND

[0003] Hot flash symptoms are physical sensations that are experienced by subjects, most commonly by women undergoing menopause. As many as 67% to 75% of women undergoing physiological or surgical menopause report experiencing hot flash symptoms. See Agarwal and Judd, 1995, *Osteoporosis: Etiology, Diagnosis, and Management*, Riggs et al., eds., Ch. 16. "Management of Menopause," pp. 351-354. Such symptoms generally include a sudden sensation of heat radiating from the face, neck, and/or chest; chills; sweating or perspiration; anxiety; tingling; and/or pressure in the head. See id. Hot flash symptoms generally do not present serious medical issues; however, approximately 10% to 15% of menopausal women experience hot flash symptoms of sufficient severity to seek medical treatment. See id.

[0004] Hot flash symptoms can also be caused as a side effect by compounds that modulate the estrogen receptor. For example, raloxifene, commonly administered in the treatment of osteoporosis, causes approximately 11% of subjects to experience hot flash symptoms of sufficient severity to discontinue therapy. See *Drug Facts and Comparisons,* 2003, Wickersham et al., eds., Wolters Lewis Health, St. Louis, Mo., pp. 225-227.

[0005] Hot flash symptoms are believed to be mediated by endocrinological changes occurring in menopausal women that likely involve downward resetting of the central thermoregulatory mechanism. See Kronenberg and Downey, 1987, *Can. J. Physol. Pharmacol.* 65:1312-1324. These changes are believed to be mediated by the hypothalamus and/or the pituitary gland. See id.

[0006] Hot flash symptoms have generally been treated with estrogen replacement therapy. See Agerwal and Judd, supra. Unfortunately, estrogen replacement therapy presents several serious side effects, including an increased risk of endometrial, ovarian, and/or breast cancer; cardiovascular disorders; hypercalcemia; glucose tolerance; depression, and hypothyroidism, among others. *See Drug Facts and Comparisons,* 2003, Wickersham et al., eds., Wolters Lewis Health, St. Louis, Mo., pp. 217-219.

[0007] Thus, there remains an unmet need for compounds that can be used to treat hot flash symptoms without the deleterious side effects presented by standard estrogen replacement therapy. In addition, rapid, inexpensive methods for determining the effects on hot flash symptoms of compounds indicated for the treatment of, for example, osteoporosis to identify therapeutic agents that do not increase the incidence of hot flash symptoms are also needed.

4. SUMMARY OF THE INVENTION

[0008] The present invention provides methods, compositions, and kits for determining the effects of candidate compounds on hot flash symptoms. Further, the invention provides methods, compositions, and kits for identifying candidate compounds that decrease hot flash symptoms. The methods and kits of the invention are based, in part, on the recognition of a correlation between the regulation of expression of certain genes by a cell and contact by the cell with compounds known to increase or decrease the incidence of hot flash symptoms. The methods and kits are described in reference to the embodiments of the invention that follow.

[0009] In certain aspects, the invention provides a method for determining the effect of a candidate compound on hot-flash symptoms that comprises contacting a first cell that expresses an estrogen receptor or estrogen related receptor with the candidate compound and determining the effect of the candidate compound on the first cell's expression of a panel of genes associated with hot flash symptoms.

[0010] In certain embodiments, the methods further comprise comparing the first cell's expression of the panel of genes associated with hot flash symptoms with a reference expression profile of the panel of genes associated with hot flash symptoms. In certain embodiments, the expression profile of the panel of genes can be the expression profile of the panel of genes following contacting the cell with a compound selected from the group consisting of estradiol, tibolone, raloxifene, and 4-hydroxy tamoxifen.

[0011] In other embodiments, the methods further comprise comparing the first cell's expression of the panel of genes associated with hot flash symptoms with a second cell's expression of the panel of genes associated with hot flash symptoms following contact with a compound that has a known effect on hot flash symptoms. The compound that has a known effect on hot flash symptoms can be any such compound known to one of skill in the art without limitations. In certain embodiments, the compound that has a known effect on hot flash symptoms can be selected from the group that consists of estradiol, tibolone, raloxifene, and 4-hydroxy tamoxifen.

[0012] In certain embodiments, the methods of the invention further comprise determining that the candidate compound decreases the incidence of hot flash symptoms.

[0013] In certain embodiments, the cell that expresses an estrogen receptor can express estrogen receptor α. In other embodiments, the cell that expresses an estrogen receptor can express estrogen receptor β. In certain embodiments, the cell that expresses the estrogen receptor can express both

estrogen receptor a and estrogen receptor β. In certain embodiments, the cell that expresses the estrogen related receptor can express estrogen related receptor α. In other embodiments, the cell that expresses the estrogen related receptor can express estrogen related receptor β. In yet other embodiments, the cell that expresses the estrogen related receptor can express estrogen related receptor γ. In still other embodiments, the cell that expresses the estrogen related receptor can express two or three estrogen related receptors, each of which is selected from the group that consists of estrogen related receptor α, estrogen related receptor β, and estrogen related receptor γ.

[0014] Any cell known by one of skill in the art to express the estrogen receptor or the estrogen related receptor, without limitation, can be used in the methods and kits of the invention. In certain embodiments, the cell that expresses the estrogen receptor or estrogen related receptor can be selected from the group consisting of a pituitary cell and a hypothalamus cell. In further embodiments, the cell that expresses the estrogen receptor can be a GH3 cell, a GH4 cell, a PR1 cell, a MtT/E-2 cell, a alphaT3-1 cell, a D12 cell, an RCF-8 cell, and a GT1-7 cell. In certain embodiments, the cell that expresses the estrogen related receptor can be selected from the group that consists of an A172 glioma cell, a MCF10a cell, a MCF12 cell, a MDA-MB-231 cell, a MDA-MB-435 cell, a MDA-MB-436 cell, a MDA-MB-468 cell, a Hs 578T cell, a BT 20 cell, a BT 474 cell, a BT 549 cell, a SKBr 3 cell, a ZR 75.1 cell, a T47D cell, and a MCF7 cell.

[0015] Any technique known by one of skill in the art to be useful in quantifying expression of a panel of genes associated with hot flash symptoms can be used in the methods of the invention, without limitation. In certain embodiments, the cell's expression of the panel of genes associated with hot flash symptoms can be quantified by a technique selected from the group of reverse transcription real time PCR, quantitative reverse transcription PCR, Northern blot assays, dot blot assays, reverse dot blot assays, RNAse protection assays, 5'-nuclease assays, reporter gene assays, branched DNA assays, bead array assays, and multiplexed array mRNA assays. In a preferred embodiment, the technique used to quantify the expression of the panel of genes associated with hot flash symptoms is a multiplexed array mRNA assay.

[0016] The panel of genes that is associated with hot flash symptoms can include any gene known by one of skill in the art to be associated with hot flash symptoms, without limitation. In certain embodiments, at least one member of the panel of genes that is associated with hot flash symptoms can be selected from the group consisting of Activin Beta E, Type II Hexokinase, Multi Drug Resistance Gene, Parvalbumin, BAD2, Prolactin, Argininosuccinate Synthetase, Ribonucleoside Reductase 1, Interleukin-18, ARL gene 4, Calpain, EST196325, CPP32, EST208064, 2-alpha-1 globin, Amiloride Binding Protein, Annexin 1, N27, HBP1, D-binding protein, FE65, Protein Kinase C type I, Glutamate Receptor subunit d1, VAP1, Protein Kinase C subspecies epsilon, EST203549, and Heat Shock Transcription Factor 1.

[0017] In a more preferred embodiment, at least one member of the panel of genes that is associated from hot flash symptoms can be selected from the group consisting of Type II Hexokinase, Multi Drug Resistance Gene, Parvalbumin, BAD2, Interleukin-18, Calpain, EST196325, Annexin 1, N27, HBP1, and Protein Kinase C subspecies epsilon.

[0018] In certain embodiments, expression of at least one member of the panel of genes can be upregulated in a cell that expresses the estrogen receptor or estrogen related receptor following contact with the candidate compound. In certain embodiments, expression of at least one member of the panel of genes can be not upregulated in a cell that expresses the estrogen receptor or estrogen related receptor following contact with the candidate compound. In certain embodiments, expression of at least one member of the panel of genes can be downregulated in a cell that expresses the estrogen receptor or estrogen related receptor following contact with the candidate compound. In certain embodiments, expression of at least one member of the panel of genes can be not downregulated in a cell that expresses the estrogen receptor or estrogen related receptor following contact with the candidate compound.

[0019] In certain embodiments, expression of Type II Hexokinase, Multi Drug Resistance Gene, Parvalbumin, BAD2, Prolactin, Interleukin-18, Calpain, and EST196325 can be upregulated in a cell that expresses the estrogen receptor or estrogen related receptor following contact with the candidate compound. In certain embodiments, expression of Annexin 1, N27, and HBP1 can be not upregulated in a cell that expresses the estrogen receptor or estrogen related receptor following contact with the candidate compound. In certain embodiments, expression of Protein Kinase C subspecies epsilon can be not downregulated in a cell that expresses the estrogen receptor or estrogen related receptor following contact with the candidate compound.

[0020] In a preferred embodiment, expression of Type II Hexokinase, Multi Drug Resistance Gene, Parvalbumin, BAD2, Prolactin, Interleukin-18, Calpain, and EST196325 are upregulated in a cell that expresses the estrogen receptor or estrogen related receptor following contact with the candidate compound; expression of Annexin 1, N27, and HBP1 is not upregulated in a cell that expresses the estrogen receptor or estrogen related receptor following contact with the candidate compound; and expression of Protein Kinase C subspecies epsilon is not downregulated in a cell that expresses the estrogen receptor or estrogen related receptor following contact with the candidate compound.

[0021] The candidate compound can be any compound known by one of skill in the art without limitation. In certain embodiments, the candidate compound can be an estrogen receptor modulator. In further embodiments, the estrogen receptor modulator can be a selective estrogen receptor modulator.

[0022] The estrogen receptor modulator can be effective to treat any disease, condition, affliction, or disorder known by one of skill in the art to be treatable with an estrogen receptor modulator, without limitation. In certain embodiments, the estrogen receptor modulator can be therapeutically effective to treat or prevent osteoporosis. In other embodiments, the selective estrogen receptor modulator can be therapeutically effective to treat or prevent post-menopausal symptoms. In still other embodiments, the selective estrogen receptor modulator can be therapeutically effective to treat or prevent a proliferative disorder.

[0023] The proliferative disorder can be any proliferative disorder known by one of skill in the art to be treatable with an estrogen receptor modulator without limitation. In certain embodiments, the proliferative disorder can be endometriosis. In other embodiments, the proliferative disorder can be cancer. In certain embodiments, the cancer can be selected from the group consisting of breast cancer, uterine cancer, ovarian cancer, cervical cancer, testicular cancer, and prostate cancer.

[0024] In another aspect, the invention provides a method for rapidly determining the effects of a plurality of compounds on hot-flash symptoms, comprising separately contacting a sample of cells that express an estrogen receptor or estrogen related receptor with each member of the plurality of compounds; and assessing the effect of each member of the plurality of compounds on each of the samples of cells' expression of a panel of genes associated with hot flash symptoms, thereby predicting the effect of each of the compounds on hot-flash symptoms.

[0025] In certain embodiments, the method further comprises comparing the expression of the panel of genes associated with hot flash symptoms by the samples of cells with a reference expression profile of the panel of genes associated with hot flash symptoms. In certain embodiments, the reference expression profile of the panel of genes can be the expression profile of the panel of genes following contacting the cell with a compound selected from the group consisting of estradiol, tibolone, raloxifene, and tamoxifen.

[0026] In other embodiments, the method further comprises comparing the expression of the panel of genes associated with hot flash symptoms by the samples of cells with the expression of the panel of genes associated with hot flash symptoms by a sample of cells following contact with a compound that has a known effect on hot flash symptoms. In certain embodiments, the compound that has a known effect on hot flash symptoms can be selected from the group that consists of estradiol, tibolone, raloxifene, and tamoxifen.

[0027] In certain embodiments, the methods further comprise determining that the compound decreases the incidence of hot flash symptoms.

[0028] In certain embodiments, the sample of cells that expresses the estrogen receptor can estrogen receptor α. In other embodiments, the sample of cells that expresses the estrogen receptor can express estrogen receptor β. In yet other embodiments, the sample of cells that expresses the estrogen receptor can express both estrogen receptor α and estrogen receptor β. In certain embodiments, the sample of cells that expresses the estrogen related receptor can express estrogen related receptor α. In other embodiments, the sample of cells that expresses the estrogen related receptor can express estrogen related receptor β. In yet other embodiments, the sample of cells that expresses the estrogen related receptor can express estrogen related receptor γ. In still other embodiments, the sample of cells that expresses the estrogen related receptor can express two or three estrogen related receptors, each of which is selected from the group that consists of estrogen related receptor α, estrogen related receptor β, and estrogen related receptor γ.

[0029] In certain embodiments, the cell that expresses the estrogen receptor can be selected from the group consisting of a pituitary cell and a hypothalamus cell. In certain embodiments, the cell that expresses the estrogen receptor can be selected from the group consisting of a GH3 cell, a GH4 cell, a PR1 cell, a MtT/E-2 cell, a alphaT3-1 cell, a D12 cell, an RCF-8 cell, and a GT1-7 cell.

[0030] In certain embodiments, the expression of the panel of genes associated with hot flash symptoms by the sample of cells can be quantified by determining the presence and amount of mRNA expressed from the panel of genes. In certain embodiments, the expression of the panel of genes associated with hot flash symptoms by the sample of cells can be quantified by a technique selected from the group of reverse transcription real time PCR, quantitative reverse transcription PCR, Northern blot assays, dot blot assays, reverse dot blot assays, RNAse protection assays, 5'-nuclease assays, reporter gene assays, branched DNA assays, bead array assays, and multiplexed array mRNA assays. In certain embodiments, the expression of the panel of genes associated with hot flash symptoms can be quantified by a multiplexed array mRNA assay.

[0031] In certain embodiments, the expression of the panel of genes associated with hot flash symptoms by the sample of cells can be quantified by determining the presence and amount of protein expressed from the panel of genes. In certain embodiments, the expression of the panel of genes associated with hot flash symptoms can be quantified by a technique selected from the group of a western blot assay, an ELISA assay, a cytokine bead array, multiplexed protein detection assays, and an immunofluorescence assay.

[0032] In certain embodiments, at least one member of the panel of genes can be selected from the group consisting of Activin Beta E, Type II Hexokinase, Multi Drug Resistance Gene, Parvalbumin, BAD2, Prolactin, Argininosuccinate Synthetase, Ribonucleoside Reductase 1, Interleukin-18, ARL gene 4, Calpain, EST196325, CPP32, EST208064, 2-alpha-1 globin, Amiloride Binding Protein, Annexin 1, N27, HBP1, D-binding protein, FE65, Protein Kinase C type I, Glutamate Receptor subunit d1, VAPI, Protein Kinase C subspecies epsilon, EST203549, and Heat Shock Transcription Factor 1. In certain embodiments, at least one member of the panel of genes can be selected from the group consisting of Type II Hexokinase, Multi Drug Resistance Gene, Parvalbumin, BAD2, Interleukin-18, Calpain, EST196325, Annexin 1, N27, HBP1, and Protein Kinase C subspecies epsilon, and expression of at least one member of the panel of genes can be upregulated in the cell following contact with the candidate compound.

[0033] In certain embodiments, at least one member of the panel of genes can be selected from the group consisting of Type II Hexokinase, Multi Drug Resistance Gene, Parvalbumin, BAD2, Interleukin-18, Calpain, EST196325, Annexin 1, N27, HBP1, and Protein Kinase C subspecies epsilon, and expression of at least one member of the panel of genes can be not upregulated in the cell following contact with the candidate compound.

[0034] In certain embodiments, at least one member of the panel of genes cam be selected from the group consisting of Type II Hexokinase, Multi Drug Resistance Gene, Parvalbumin, BAD2, Prolactin, Interleukin-18, Calpain, EST196325, Annexin 1, N27, HBP1, and Protein Kinase C subspecies epsilon, and expression of at least one member of the panel of genes can be downregulated in the cell following contact with the candidate compound.

[0035] In certain embodiments, at least one member of the panel of genes can be selected from the group consisting of Type II Hexokinase, Multi Drug Resistance Gene, Parvalbumin, BAD2, Prolactin, Interleukin-18, Calpain, EST196325, Annexin 1, N27, HBP1, and Protein Kinase C subspecies epsilon, and expression of at least one member of the panel of genes can be not downregulated in the cell following contact with the candidate compound.

[0036] In certain embodiments, the panel of genes comprises Type II Hexokinase, Multi Drug Resistance Gene, Parvalbumin, BAD2, Prolactin, Interleukin-18, Calpain, EST196325, Annexin 1, N27, and HBP1; expression of Type II Hexokinase, Multi Drug Resistance Gene, Parvalbumin, BAD2, Prolactin, Interleukin-18, Calpain, and EST196325 can be upregulated in the cell following contact with the candidate compound; expression of Annexin 1, N27, and HBP1 can be not upregulated in the cell following contact with the candidate compound; and expression of Protein Kinase C subspecies epsilon can be not downregulated in the cell following contact with the candidate compound.

[0037] In another aspect, the invention provides kits for determining the effect of a compound on hot flash symptoms. The kits generally comprise at least one primer or probe that can be used to detect the presence and amount of an expression product of a member of a panel of genes associated with hot flash symptoms. In certain embodiments, the primer or probe can be used to detect an mRNA expressed from a member of a panel of genes associated with hot flash symptoms. In other embodiments, the primer or probe can be used to detect a protein expressed from a member of a panel of genes associated with hot flash symptoms.

[0038] In a preferred embodiment, the kits can comprise at least one gene-specific nuclease protection probe that is specific for a member of a panel of genes associated with hot flash symptoms and that can be directly or indirectly detectable; and a surface having multiple spatially discrete regions, at least two of which regions are substantially identical, and wherein the regions are adapted to specifically bind to the gene-specific protection probe(s).

[0039] In yet another aspect, the invention provides compositions suitable for determining the effect of a compound on hot flash symptoms. The compositions may also be used in the methods and kits of the invention. The compositions of the invention generally comprise at least one primer or probe that can be used to detect the presence and amount of an expression product of a member of a panel of genes associated with hot flash symptoms and a suitable buffer, diluent, or excipient. The member of the panel of genes can be any member of a panel of genes associated with hot flash symptoms known by one of skill in the art without limitation.

[0040] In certain embodiments, the primer or probe can be used to detect an mRNA expressed from a member of a panel of genes associated with hot flash symptoms. In other embodiments, the primer or probe can be used to detect a protein expressed from a member of a panel of genes associated with hot flash symptoms. In a preferred embodiment, the composition comprises a plurality of gene-specific nuclease protection probes, wherein each member of the plurality of gene-specific nuclease protection probes hybridizes under stringent conditions to an mRNA expressed from a member of a panel of genes associated with hot flash symptoms.

[0041] In still another aspect, the invention provides arrays useful for the identification of the effect of a plurality of compounds on hot flash symptoms. In certain embodiments, the array can comprise a non-porous surface; and a plurality of different oligonucleotides connected with the surface, wherein at least one of the oligonucleotides hybridizes under stringent conditions to a member of a panel of genes associated with hot flash symptoms, and wherein each of the different oligonucleotides is connected with the surface in a different predetermined region of the surface. The member of the panel of genes can be any member of a panel of genes associated with hot flash symptoms known by one of skill in the art, without limitation.

5. BRIEF DESCRIPTION OF THE FIGURES

[0042] **FIG. 1A** presents the effects of estradiol, raloxifene, tibolone, and 4-hydroxy tamoxifen on expression of BAD2 and MDR in GH3 cells;

[0043] **FIG. 1B** presents the effects of estradiol, raloxifene, tibolone, and 4-hydroxy tamoxifen on expression of Prolactin and Parvalbumin in GH3 cells;

[0044] **FIG. 1C** presents the effects of estradiol, raloxifene, tibolone, and 4-hydroxy tamoxifen on expression of HKII and Calpain in GH3 cells;

[0045] **FIG. 1D** presents the effects of estradiol, raloxifene, tibolone, and 4-hydroxy tamoxifen on expression of Il-18 and Annexin 1 in GH3 cells; and

[0046] **FIG. 1E** presents the effects of estradiol, raloxifene, tibolone, and 4-hydroxy tamoxifen on expression of N27 (Vdup1) and Protein Kinase C, epsilon subspecies in GH3 cells.

6. DETAILED DESCRIPTION OF EMBODIMENTS OF THE INVENTION

[0047] The present invention provides methods, kits, compositions, and arrays for determining the effect of candidate compounds on hot flash symptoms. The methods, kits, compositions, and arrays are described according to the various embodiments of the invention presented in detail below.

6.1. Definitions

[0048] The terms "reference compound(s),""compound(s) known to affect hot flash symptoms," and "compound(s) with a known effect on hot flash symptoms" are used interchangeably and generally refer to compounds that are known by one of skill in the art to increase or decrease the incidence of hot flash symptoms when the compounds are administered to a subject. Exemplary reference compounds include estradiol, tibolone, raloxifene, and 4-hydroxy tamoxifen.

[0049] The terms "candidate compound" or "candidate compounds" refer to one or more compounds whose effects on hot flash symptoms are to be determined according to the methods of the invention. The candidate compound can be any compound known by one of skill in the art without limitation. The candidate compound can be derived from

any source known to one of skill in the art without limitation. For example, and not by way of limitation, the candidate compound can be organic or inorganic; polar or non-polar; neutrally charged, positively charged, negatively charged, or zwitterionic; a small organinc molecule or a large macromolecule, etc. Candidate compounds suitable for the methods of the invention can be obtained from any commercial source, including Aldrich (1001 West St. Paul Ave., Milwaukee, Wis. 53233), Sigma Chemical (P.O. Box 14508, St. Louis, Mo. 63178), Fluka Chemie AG (Industriestrasse 25, CH-9471 Buchs, Switzerland (Fluka Chemical Corp. 980 South 2nd Street, Ronkonkoma, N.Y. 11779)), Eastman Chemical Company, Fine Chemicals (P.O Box 431, Kingsport, Tenn. 37662), Boehringer Mannheim GmbH (Sandhofer Strasse 116, D-68298 Mannheim), Takasago (4 Volvo Drive, Rockleigh, N.J. 07647), SST Corporation (635 Brighton Road, Clifton, N.J. 07012), Ferro (111 West Irene Road, Zachary, La. 70791), Riedel-deHaen Aktiengesellschaft (P.O. Box D-30918, Seelze, Germany), PPG Industries Inc., Fine Chemicals (One PPG Place, 34th Floor, Pittsburgh, Pa. 15272). Further, the effect of any kind of natural product on expression of genes associated with hot flash symptoms may be determined according to the methods of the invention, including microbial, fungal or plant extracts.

[0050] The term "hot flash symptom(s)" refers to one or more symptoms commonly experienced by subjects, typically women, typically at menopause. Hot flash symptoms can include, but are not limited to, a sudden sensation of heat radiating from the face, neck, and/or chest; chills; sweating or perspiration; anxiety; tingling; and/or pressure in the head. Hot flash symptoms can be experienced by subjects for short, e.g., seconds, or long, e.g., hours, durations.

[0051] The term "upregulated" refers to an increase in the expression of one or more genes associated with hot flash symptoms in a cell following contact of the cell with a compound that has an known or unknown effect on hot flash symptoms.

[0052] The term "downregulated" refers to a decrease in the expression of one or more genes associated with hot flash symptoms in a cell following contact of the cell with a compound that has an known or unknown effect on hot flash symptoms.

[0053] The term "panel of genes associated with hot flash symptoms" refers to a plurality of genes, expression of each of which is modulated by a compound that has a known effect on hot flash symptoms. A panel of genes associated with hot flash symptoms can comprise as few as two genes or as many as 30, or more. In certain embodiments, the panel of genes associated with hot flash symptoms comprises 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 18, 20, 22, or 24 genes associated with hot flash symptoms. In a preferred embodiment, the panel of genes associated with hot flash symptoms comprises 10 genes associated with hot flash symptoms.

[0054] The term "high throughput method for determining regulation of gene expression" refers to any method known by one of skill in the art without limitation suitable for determining the effects of a large number of candidate compounds on regulation of expression of genes. Such methods include, for example, but not by way of limitation, the multiplexed array mRNA assays, differential PCR, restriction mediated differential display, AFLP-based transcript profiling, serial expression of gene analysis, Massive Parallel Signature Sequencing, dot blot assays, reverse dot blot assays, and bead array-based assays.

[0055] The term "reference expression profile," as used herein, refers to the profile of expression of a panel of genes associated with hot flash symptoms in a cell following contact of the cell with a compound that has a known effect on hot flash symptoms. The reference expression profile can be the profile of expression of genes associated with hot flash symptoms in a cell following contact of the cell with any compound with a known effect on hot flash symptoms known by one of skill in the art without limitation. In certain embodiments, the reference expression profile is the profile of expression of genes associated with hot flash symptoms in a cell following contact of the cell with estradiol, tibolone, raloxifene, or 4-hydroxy tamoxifen.

[0056] As used herein, the terms "nucleic acid,""nucleotide,""polynucleotide" and "oligonucleotide" refer to primers, probes, oligomer fragments to be detected, oligomer controls and unlabeled blocking oligomers and is generic to polydeoxyribonucleotides (containing 2-deoxy-D-ribose), to polyribonucleotides (containing D-ribose), and to any other N-glycoside of a purine or pyrimidine base, or modified purine or pyrimidine bases.

[0057] A nucleic acid, nucleotide, polynucleotide or oligonucleotide can comprise phosphodiester linkages or modified linkages including, but not limited to phosphotriester, phosphoramidate, siloxane, carbonate, carboxymethylester, acetamidate, carbamate, thioether, bridged phosphoramidate, bridged methylene phosphonate, phosphorothioate, methylphosphonate, phosphorodithioate, bridged phosphorothioate or sulfone linkages, and combinations of such linkages.

[0058] A nucleic acid, nucleotide, polynucleotide or oligonucleotide can comprise the five biologically occurring bases (adenine, guanine, thymine, cytosine and uracil) and/or bases other than the five biologically occurring bases. These bases may serve a number of purposes, e.g., to stabilize or destabilize hybridization; to promote or inhibit probe degradation; or as attachment points for detectable moieties or quencher moieties. For example, a polynucleotide of the invention can contain one or more modified, non-standard, or derivatized base moieties, including, but not limited to, N^6-methyl-adenine, N^6-tert-butyl-benzyl-adenine, imidazole, substituted imidazoles, 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxymethyl)uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-methyladenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acidmethylester, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, 2,6-diaminopurine, and 5-propynyl pyrimidine. Other examples of modified, non-standard, or derivatized base moieties may be found in U.S. Pat. Nos.

6,001,611, 5,955,589, 5,844,106, 5,789,562, 5,750,343, 5,728,525, and 5,679,785, each of which is incorporated herein by reference in its entirety.

[0059] Furthermore, a nucleic acid, nucleotide, polynucleotide or oligonucleotide can comprise one or more modified sugar moieties including, but not limited to, arabinose, 2-fluoroarabinose, xylulose, and hexose.

[0060] It is not intended that the present invention be limited by the source of a nucleic acid, nucleotide, polynucleotide or oligonucleotide. A nucleic acid, nucleotide, polynucleotide or oligonucleotide can be from a human or non-human mammal, or any other organism, or derived from any recombinant source, synthesized in vitro or by chemical synthesis. A nucleic acid, nucleotide, polynucleotide or oligonucleotide may be DNA, RNA, cDNA, DNA-RNA, locked nucleic acid (LNA), peptide nucleic acid (PNA), a hybrid or any mixture of the same, and may exist in a double-stranded, single-stranded or partially double-stranded form. A nucleic acid may also be a derivative nucleic acid as described in U.S. Pat. No. 5,696,248, which is hereby incorporated by reference in its entirety. The nucleic acids of the invention include both nucleic acids and fragments thereof, in purified or unpurified forms, including genes, chromosomes, plasmids, the genomes of biological material such as microorganisms, e.g., bacteria, yeasts, viruses, viroids, molds, fungi, plants, animals, humans, and the like.

[0061] There is no intended distinction in length between the terms nucleic acid, nucleotide, polynucleotide and oligonucleotide, and these terms will be used interchangeably. These terms include double- and single-stranded DNA, as well as double- and single-stranded RNA, as appropriate for the context.

[0062] The term "primer" refers to an oligonucleotide which is capable of acting as a point of initiation of polynucleotide synthesis along a template nucleic acid strand when placed under conditions that permit synthesis of a primer extension product that is complementary to the template strand. The primer can be obtained from a recombinant source, as in a purified restriction fragment, or produced synthetically. Primer extension conditions typically include the presence of four different deoxyribonucleoside triphosphates and an agent with polymerization activity such as DNA polymerase or reverse transcriptase, in a suitable buffer (a "buffer" can include substituents which are cofactors, or which affect pH, ionic strength, etc.), and at a suitable temperature. The primer is preferably single-stranded for maximum efficiency in amplification.

[0063] The term "hybridize" refers to binding of a single-stranded nucleic acid or a locally single-stranded region of a double-stranded nucleic acid to another single-stranded nucleic acid or a locally single-stranded region of a double-stranded nucleic acid having a complementary sequence. As one of skill in the art is aware, it is not necessary for two nucleic acid strands to be entirely complementary to hybridize to each other. Depending on the hybridization conditions, a nucleic acid can hybridize to its complement even if there are few, some, or many mismatches, deletions, or additions in one or both strands. In certain embodiments, the primers and probes of the invention can hybridize to an at least partially complementary sequence under stringent conditions, as defined below.

[0064] The terms "stringent" or "stringent conditions," as used herein, denote hybridization conditions of low ionic strength and high temperature, as is well known in the art; see for example Maniatis et al., 1989, Molecular Cloning: A Laboratory Manual, 2d Edition; Current Protocols in Molecular Biology, 1988, ed. Ausubel et al., J. Wiley & Sons publ., New York, and Tijssen, 1993, Techniques in Biochemistry and Molecular Biology-Hybridization with Nucleic Acid Probes, "Overview of principles of hybridization and the strategy of nucleic acid assays," each of which is hereby incorporated by reference. Generally, stringent conditions are selected to be about 5-30° C. lower than the thermal melting point (Tm) for the specified sequence at a defined ionic strength and pH. Alternatively, stringent conditions are selected to be about 5-15° C. lower than the thermal melting point (Tm) for the specified sequence at a defined ionic strength and pH. The Tm is the temperature (under defined ionic strength, pH and nucleic acid concentration) at which 50% of the probes complementary to the target hybridize to the target sequence at equilibrium (as the target sequences are present in excess, at Tm, 50% of the probes are occupied at equilibrium). For example, stringent hybridization conditions can be those in which the salt concentration is less than about 1.0 M sodium (or other salts) ion, typically about 0.01 to about 1 M sodium ion concentration at about pH 7.0 to about pH 8.3 and the temperature is at least about 25° C. for short probes (e.g., 10 to 50 nucleotides) and at least about 55° C. for long probes (e.g., greater than 50 nucleotides). Stringent conditions may also be modified with the addition of hybridization destabilizing agents such as formamide.

[0065] The term "highly stringent conditions" is meant to refer to hybridization of a strand of a nucleic acid to a complementary strand of nucleic acid under conditions that permit specific association between the two strands. The nucleic acid strands need not be entirely complementary to hybridize under highly stringent conditions; one of ordinary skill in the art will recognize that nucleic acids can hybridize to each other under highly stringent conditions notwithstanding a certain amount of mismatches, insertions, deletions, etc. One example of "highly stringent conditions" for hybridization of nucleic acids is hybridization in a buffer that comprises 0.5 M NaHPO4, 7% sodium dodecyl sulfate (SDS), 1 mM EDTA at 65° C., and washing in 0.1×SSC/0.1% SDS at 68° C. (Ausubel F. M. et al., eds., 1989, Current Protocols in Molecular Biology, Vol. 1, at p. 2.10.3). Another example of "highly stringent conditions" that can be used for hybridization of an oligonucleotide to another nucleic acid comprises washing in 6×SSC/0.05% sodium pyrophosphate at 37° C. (for 14 base oligos), 48° C. (for 17 base oligos), 55° C. (for 20 base oligos), and 60° C. (for 23 base oligos).

[0066] The term "moderately stringent conditions" is meant to refer to hybridization of a strand of a nucleic acid to a complementary strand of nucleic acid under conditions that permit specific association between the two strands, wherein the strands are less complementary than strands that will associate under highly specific conditions. As one of skill in the art is well aware, "moderately stringent conditions" allow the specific association of nucleic acids that contain sufficient mismatches, insertions, deletions, etc. to prevent specific association under highly stringent conditions, but nonetheless retain sufficient sequence complementarity to specifically associate. One example of "moderately stringent conditions" comprises washing in 0.2×SSC/0.1% SDS at 42° C. (Ausubel et al., 1989, supra).

[0067] The "complement" of a nucleic acid sequence, as used herein, refers to an oligonucleotide which, when aligned with the nucleic acid sequence such that the 5' end of one sequence is paired with the 3 ' end of the other, is in anti-parallel association. The complement of a nucleic acid sequence need not exactly match every nucleotide of the sequence; stable duplexes may contain mismatched base pairs, unmatched bases, insertions, or deletions. Those skilled in the art of nucleic acid technology can determine duplex stability by empirically considering a number of variables including, for example, the length of the oligonucleotide, base composition and sequence of the oligonucleotide, ionic strength, and incidence of mismatched base pairs.

[0068] Stability of a nucleic acid duplex is measured by the melting temperature, or "T_m." The T_m of a particular nucleic acid duplex under specified conditions is the temperature at which half of the potential base pairs are disassociated.

[0069] The term "detectable moiety" as used herein refers to any atom or molecule which can be used to provide a detectable, quantifiable signal, and which can be attached to a nucleic acid or protein. Detectable moieties may provide signals detectable by fluorescence, radioactivity, colorimetry, gravimetry, X-ray diffraction or absorption, magnetism, enzymatic activity, and the like.

[0070] The term "fluorescent moiety" as used herein refers to a chemical moiety that can emit light under conditions appropriate for the particular moiety. Typically, a particular fluorescent moiety can emit light of a particular wavelength following absorbance of light of shorter wavelength. The wavelength of the light emitted by a particular fluorescent moiety is characteristic of that moiety. Thus, a particular fluorescent moiety can be detected by detecting light of an appropriate wavelength following excitation of the fluorescent moiety with light of shorter wavelength. Examples of fluorescent moieties that can be used in the methods and compositions of the present invention include, but are not limited to, fluorescein-family dyes, polyhalofluorescein-family dyes, hexachlorofluorescein-family dyes, coumarin-family dyes, rhodamine-family dyes, cyanine-family dyes, oxazine-family dyes, thiazine-family dyes, squaraine-family dyes, chelated lanthanide-family dyes, and BODIPY®-family dyes.

[0071] The term "control assay" as used herein refers to a reaction performed as described below with a compound that has a known effect on regulation of genes associated with hot flash symptoms and/or a known effect on hot flash symptoms. The amount of signal emitted by such a reaction can be compared to a reaction performed using a candidate compound to determine the effect of the candidate compound on regulation of genes associated with hot flash symptoms and/or a known effect on hot flash symptoms.

[0072] To determine "percent complementarity" or "percent identity" of two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in the sequence of a first nucleic acid sequence for optimal alignment with a second nucleic acid sequence). The nucleotides at corresponding nucleotide positions are then compared. When a position in the first sequence is occupied by a complementary nucleotide as the corresponding position in the second sequence, then the molecules are complementary at that position. Likewise, when a position in the first sequence is occupied by the same nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position. The percent complementarity (or percent identity) between the two sequences is a function of the number of complementary positions (or identical positions) shared by the sequences divided by the total number of positions compared (i.e., % complementarity=number of complementary overlapping positions/total number of positions of the shorter nucleotide×100%; and % identity=number of identical overlapping positions/total number of positions of the shorter nucleotide×100%).

[0073] The determination of percent identity between two sequences can also be accomplished using a mathematical algorithm. A preferred, non-limiting example of a mathematical algorithm utilized for the comparison of two sequences is the algorithm of Karlin and Altschul, 1990, *Proc. Natl. Acad. Sci. U.S.A.* 87:2264-2268, modified as in Karlin and Altschul, 1993, Proc. Natl. Acad. Sci. U.S.A. 90:5873-5877. Such an algorithm is incorporated into the NBLAST program of Altschul et al., 1990, *J. Mol. Biol.* 215:403.

[0074] The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology, microbiology and recombinant DNA techniques, which are within the skill of the art. Such techniques are explained fully in the literature. See, e.g., Sambrook et al., 2001, *Molecular Cloning: A Laboratory Manual*, Third Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York; Oligonucleotide Synthesis (M. J. Gait, ed., 1984); *Nucleic Acid Hybridization* (B. D. Hames & S. J. Higgins, eds., 1984); *A Practical Guide to Molecular Cloning* (B. Perbal, 1984); and a series, *Methods in Enzymology* (Academic Press, Inc.).

6.2. Methods of Determining the Effect of a Candidate Compound on Hot Flash Symptoms

[0075] The present invention provides a method for determining the effect of a candidate compound on hot flash symptoms. The method generally comprises exposing a cell that expresses an estrogen receptor or estrogen related receptor to a candidate compound and determining the effect of the candidate compound on the cell's expression of a panel of genes associated with hot flash symptoms. In certain embodiments, the method can be used to identify the effect of a single compound on the expression of a panel of genes associated with hot flash symptoms. In other embodiments, the methods can identify the effect of more than one compound on the expression of a panel of genes associated with hot flash symptoms. Thus, the method of the invention can be used to achieve at least two major objectives: the method can be used to identify compounds that decrease the incidence of hot flash symptoms, and the method can be used to determine whether compounds that have some other, useful effect, e.g., compounds effective to treat osteoporosis or breast cancer, undesirably increase the incidence of hot flash symptoms.

6.2.1. Compounds Having a Known Effect on Hot Flash Symptoms

[0076] The present invention is based, in part, on the discovery of a correlation between regulation of expression

of certain genes in a cell that expresses the estrogen receptor or estrogen related receptor and contact of the cell by compounds that have a known effect on hot flash symptoms. Thus, based upon the disclosure of the present invention, one of skill in the art can determine the effect of a candidate compound on hot flash symptoms by contacting a cell with the candidate compound, and determining the effect of the candidate compound on the expression of genes associated with hot flash symptoms. The effects of the candidate compound on hot flash symptoms can be determined by comparing the expression of the panel of genes with a reference expression profile generated by a compound or compounds that has a known effect on hot flash symptoms.

[0077] Any compound, without limitation, that is known by one of skill in the art to have an effect on hot flash symptoms when administered to a subject can be used as a reference compound in the methods of the invention. The reference compound can either increase or decrease the incidence of hot flash symptoms. Gene expression in a cell that expresses the estrogen receptor or estrogen related receptor can be assessed in the presence and absence of the reference compound. Genes that are differentially expressed following contact with a reference compound can thus be identified. The expression of such genes can be upregulated or downregulated following such contact with a reference compound.

[0078] Following identification of genes that are upregulated or downregulated by compounds that have a known effect on hot flash symptoms, the effects of the reference compound on gene expression and on hot flash symptoms can be correlated. For example, estradiol and tibolone are known to decrease the incidence of hot flash symptoms when administered to a subject, while raloxifene and 4-hydroxy tamoxifen are known to increase the incidence of hot flash symptoms when administered to a subject. Thus, a candidate compound that has a similar effect on gene expression to estradiol or tibolone is likely to decrease the incidence of hot flash symptoms when administered to a subject. Conversely, a candidate compound that has a similar effect on gene expression to raloxifene or 4-hydroxy tamoxifen is likely to decrease the incidence of hot flash symptoms when administered to a subject. Any other compound whose effect on hot flash symptoms is known to one of skill in the art can be similarly used in the methods of the invention.

[0079] Accordingly, in certain aspects, the present invention provides methods of determining the effect of a candidate compound or a library of candidate compounds on hot flash symptoms. The methods generally comprise determining the effect of the candidate compound on expression of a panel of genes associated with hot flash symptoms.

[0080] In certain embodiments, the methods further comprise comparing the expression of the panel of genes associated with hot flash symptoms with a reference expression profile of the panel of genes associated with hot flash symptoms. In certain embodiments, the reference expression profile of the panel of genes associated with hot flash symptoms can be the expression profile of the panel of genes following contact of a cell with a compound selected from the group consisting of estradiol, tibolone, raloxifene, and 4-hydroxy tamoxifen. In other embodiments, the reference expression profile of the panel of genes associated with hot flash symptoms can be the expression profile of the panel of genes following contact of a cell with estradiol. In still other embodiments, the reference expression profile of the panel of genes associated with hot flash symptoms can be the expression profile of the panel of genes following contact of a cell with tibolone. In yet other embodiments, the reference expression profile of the panel of genes associated with hot flash symptoms can be the expression profile of the panel of genes following contact of a cell with raloxifene. In still other embodiments, the reference expression profile of the panel of genes associated with hot flash symptoms can be the expression profile of the panel of genes following contact of a cell with 4-hydroxy tamoxifen.

[0081] In other embodiments, the method further comprises comparing the expression profile of a panel of genes associated with hot flash symptoms in a cell following contact with a candidate compound with the expression profile of the panel of genes associated with hot flash symptoms in a cell following contact with a reference compound. In certain embodiments, the reference compound can be selected from the group that consists of estradiol, tibolone, raloxifene, and 4-hydroxy tamoxifen. In other embodiments, the reference compound can be estradiol. In still other embodiments, the reference compound can be tibolone. In yet other embodiments, the reference compound can be raloxifene. In still other embodiments, the reference compound can be 4-hydroxy tamoxifen.

6.2.2. Genes Associated with Hot Flash Symptoms and Their Regulation By Compounds Having a Known Effect on Hot Flash Symptoms

[0082] The present invention provides a large number of genes that are differentially expressed following contact with a compound that has a known effect on hot flash symptoms. The effect on expression of such genes in a cell following contact with a candidate compound can be compared to the effect on expression of these genes following contact with a reference compound, thereby determining the effect of the candidate compound on hot flash symptoms. Genes that can are differentially expressed following contact with a compound known to affect hot flash symptoms are described below.

[0083] The expression of any gene known by one of skill in the art to be associated with hot flash symptoms, without limitation, can be assessed in the methods of the invention. The present invention provides numerous such genes as described in detail, below. Further, additional genes that are associated with hot flash symptoms can readily be identified by one of skill in the art according to the disclosure of the present invention. For example, a cell that expresses the estrogen receptor or estrogen related receptor can be contacted with a reference compound known to affect hot flash symptoms. The overall expression of genes in the cell following contact with the reference compound can be compared to the overall expression of genes in a similar cell that has not been contacted with the reference compound.

[0084] The expression of such genes to identify genes that are differentially expressed following contact with a compound with a known effect on hot flash symptoms can be monitored by any convenient method known by one of skill in the art. Most advantageously, the expression of many genes, e.g., thousands of genes, is monitored at once using a high throughput method for assessing expression of genes in the presence and absence of compounds known to affect hot flash symptoms.

[0085] Using, for example, a gene array from Affymetrix, Inc. (Sunnyvale, Calif.), genes that are differentially expressed following contact with the reference compound can be identified. Affymetrix gene arrays, and methods of making and using such arrays, are described in, for example, U.S. Pat. Nos. 6,551,784, 6,548,257, 6,505,125, 6,489,114, 6,451,536, 6,410,229, 6,391,550, 6,379,895, 6,355,432, 6,342,355, 6,333,155, 6,308,170, 6,291,183, 6,287,850, 6,261,776, 6,225,625, 6,197,506, 6,168,948, 6,156,501, 6,141,096, 6,040,138, 6,022,963, 5,919,523, 5,837,832, 5,744,305, 5,834,758, and 5,631,734, each of which is hereby incorporated by reference in its entirety. In addition, Ausubel et al., eds., *Current Protocols in Molecular Biology,* 2002, Vol. 4, Unit 25B, Ch. 22, which is hereby incorporated by reference in its entirety, provides further guidance on construction and use of a gene array for identifying genes of interest, e.g., genes associated with hot flash symptoms, that are differentially expressed in different samples of cells.

[0086] Other techniques suitable for such analysis include differential PCR, restriction mediated differential display, AFLP-based transcript profiling, serial expression of gene analysis ("SAGE"), and Massive Parallel Signature Sequencing ("MPSS"). These other techniques and protocols for performing the techniques are well-known in the art and are described in Ausubel et al., eds., *Current Protocols in Molecular Biology,* 2002, Vol. 4, Unit 25B, Ch. 3-6, except for MPSS, which is described in Brenner et al., 2000, *Nat. Biochem.* 18:630-634. Each of these references is hereby incorporated by reference in its entirety. Expression of genes that are identified in such a manner can be assessed to determine the effect of candidate compounds on hot flash symptoms.

[0087] Using the above-described methods, a number of genes have been identified that are associated with hot flash symptoms. TABLE 1, below, presents a number of examples of such genes and further, provides examples of effects on regulation of expression of these genes in a cell following contact with reference compounds that have known effects on hot flash symptoms.

TABLE 1

Gene Name	Abbreviation or Alternative Name	Accession Number	Effect of Estradiol on Expression	Effect of Tibolone on Expression	Effect of Raloxifene on Expression	Effect of 4-hydroxy tamoxifen on Expression
Activin Beta E SEQ ID NO: 1		AF089825	Upregulated	Upregulated	Down-regulated	Down-regulated
Type II Hexokinase SEQ ID NO: 2	HKII	D26393	Upregulated	Upregulated	Down-regulated	Down-regulated
Multi Drug Resistance Gene SEQ ID NO: 3	MDR	M81855	Upregulated	Upregulated	Down-regulated	Down-regulated
Parvalbumin SEQ ID NO: 4		AF022935	Upregulated	Upregulated	Down-regulated	Down-regulated
Protein Tyrosine Phosphatase BAD2 SEQ ID NO: 5	BAD2	U02553	Upregulated	Upregulated	Down-regulated	Down-regulated
Prolactin SEQ ID NO: 6		AI175539	Upregulated	Upregulated	Down-regulated	Down-regulated
Argininosuccinate Synthetase SEQ ID NO: 7		X12459	Upregulated	Upregulated	Down-regulated	Down-regulated
Ribonucleoside Reductase Nuclear Receptor SEQ ID NO: 8	RNR-1	L08595	Upregulated	Upregulated	No Significant Effect	No Significant Effect
Interleukin-18 SEQ ID NO: 9	IL-18	AJ222813	Upregulated	Upregulated	No Significant Effect	No Significant Effect
ARL gene 4 SEQ ID NO: 10		X77235	Upregulated	Upregulated	No Significant Effect	No Significant Effect
Calpain SEQ ID NO: 11		D14478	Upregulated	Upregulated	No Significant Effect	No Significant Effect
EST196325 SEQ ID NO: 12		AA892522	Upregulated	Upregulated	No Significant Effect	No Significant Effect
Interleukin-1 β-converting enzyme-related protease CPP32 SEQ ID NO: 13	CPP32	U84410	Upregulated	Upregulated	No Significant Effect	No Significant Effect
EST208064 SEQ ID NO: 14		AI013389	Upregulated	Upregulated	No Significant Effect	No Significant Effect
Amiloride Binding Protein SEQ ID NO: 15		X73911	Down-regulated	Down-regulated	No Significant Effect	No Significant Effect
2-alpha-1 globin SEQ ID NO: 16		X56325	Upregulated	Upregulated	No Significant Effect	No Significant Effect

TABLE 1-continued

Gene Name	Abbreviation or Alternative Name	Accession Number	Effect of Estradiol on Expression	Effect of Tibolone on Expression	Effect of Raloxifene on Expression	Effect of 4-hydroxy tamoxifen on Expression
Annexin 1 SEQ ID NO: 17	EST 21795	AI171962	No Significant Effect	No Significant Effect	Upregulated	Upregulated
N27 SEQ ID NO: 18	EST 207724	AI014169	No Significant Effect	No Significant Effect	Upregulated	Upregulated
HMG-box containing protein 1 SEQ ID NO: 19	HBP1	U09551	No Significant Effect	No Significant Effect	Upregulated	Upregulated
D-binding protein SEQ ID NO: 20		J03179	No Significant Effect	No Significant Effect	Upregulated	Upregulated
FE65 adaptor protein interacting with beta-amyloid precursor protein intracellular domain SEQ ID NO: 21	FE65	X60469	No Significant Effect	No Significant Effect	Upregulated	No Significant Effect
Protein Kinase C type I SEQ ID NO: 22	PKC type I	M13707	No Significant Effect	No Significant Effect	Upregulated	Upregulated
Glutamate Receptor (d1 subunit) SEQ ID NO: 23		U08255	No Significant Effect	No Significant Effect	Down-regulated	No Significant Effect
Vesicle Associated Protein VAP1 SEQ ID NO: 24	VAP1	AF034582	No Significant Effect	No Significant Effect	Down-regulated	No Significant Effect
Protein Kinase C subspecies epsilon SEQ ID NO: 25		M18331	No Significant Effect	No Significant Effect	Down-regulated	No Significant Effect
EST 203549 SEQ ID NO: 26		AI009098	No Significant Effect	No Significant Effect	Down-regulated	No Significant Effect
Heat Shock Transcription Factor 1 SEQ ID NO: 27		X83094	No Significant Effect	No Significant Effect	Down-regulated	No Significant Effect

[**0088**] As shown in TABLE 1, the present invention provides a number of genes that are differentially expressed following contact with compounds that have known effects on hot flash symptoms. By comparing the effects of the reference compounds on expression of genes associated with hot flash symptoms to the effect of a candidate compound on expression of these genes, the effect of the candidate compound on hot flash symptoms can be determined.

[**0089**] Accordingly, in certain aspects, the invention provides methods of determining the effect of a candidate compound on hot flash symptoms that comprise determining the effect of the candidate compound on expression of a panel of genes associated with hot flash symptoms.

[**0090**] In certain embodiments, at least one member of the panel of genes that is associated with hot flash symptoms can be selected from the group consisting of Activin Beta E, Type II Hexokinase, Multi Drug Resistance Gene, Parvalbumin, BAD2, Prolactin, Argininosuccinate Synthetase, Ribonucleoside Reductase 1, Interleukin-18, ARL gene 4, Calpain, EST196325, CPP32, EST208064, 2-alpha-1 globin, Amiloride Binding Protein, Annexin 1, N27, HBP1, D-binding protein, FE65, Protein Kinase C type I, Glutamate Receptor subunit d1, VAP1, Protein Kinase C subspecies epsilon, EST203549, and Heat Shock Transcription Factor 1. In other embodiments, more than one member of the panel of genes that is associated with hot flash symptoms can be selected from the group consisting of Activin Beta E, Type II Hexokinase, Multi Drug Resistance Gene, Parvalbumin, BAD2, Prolactin, Argininosuccinate Synthetase, Ribonucleoside Reductase 1, Interleukin-18, ARL gene 4, Calpain, EST196325, CPP32, EST208064, 2-alpha-1 globin, Amiloride Binding Protein, Annexin 1, N27, HBP1, D-binding protein, FE65, Protein Kinase C type I, Glutamate Receptor subunit d1, VAP1, Protein Kinase C subspecies epsilon, EST203549, and Heat Shock Transcription Factor 1. In still other embodiments, each member of the panel of genes that is associated with hot flash symptoms can be selected from the group consisting of Activin Beta E, Type II Hexokinase, Multi Drug Resistance Gene, Parvalbumin, BAD2, Prolactin, Argininosuccinate Synthetase, Ribonucleoside Reductase 1, Interleukin-18, ARL gene 4, Calpain, EST196325, CPP32, EST208064, 2-alpha-1

globin, Amiloride Binding Protein, Annexin 1, N27, HBP1, D-binding protein, FE65, Protein Kinase C type I, Glutamate Receptor subunit d1, VAP1, Protein Kinase C subspecies epsilon, EST203549, and Heat Shock Transcription Factor 1.

[0091] In more preferred embodiments, at least one member of the panel of genes associated from hot flash symptoms can be selected from the group consisting of Type II Hexokinase, Multi Drug Resistance Gene, Parvalbumin, BAD2, Interleukin-18, Calpain, EST196325, Annexin 1, N27, HBP1, and Protein Kinase C subspecies epsilon. In still more preferred embodiments, more than one member of the panel of genes associated from hot flash symptoms can be elected from the group consisting of Type II Hexokinase, Multi Drug Resistance Gene, Parvalbumin, BAD2, Interleukin-18, Calpain, EST196325, Annexin 1, N27, HBP1, and Protein Kinase C subspecies epsilon. In yet more preferred embodiments, each member of the panel of genes associated from hot flash symptoms can be selected from the group consisting of Type II Hexokinase, Multi Drug Resistance Gene, Parvalbumin, BAD2, Interleukin-18, Calpain, EST196325, Annexin 1, N27, HBP1, and Protein Kinase C subspecies epsilon.

[0092] In certain embodiments, the panel of genes comprises two genes associated with hot flash symptoms. In other, more preferred, embodiments, the panel of genes comprises four genes associated with hot flash symptoms. In other, even more preferred, embodiments, the panel of genes comprises five genes associated with hot flash symptoms. In yet other, even more preferred, embodiments, the panel of genes comprises eight genes associated with hot flash symptoms. In the most preferred embodiment, the panel of genes comprises ten genes associated with hot flash symptoms.

[0093] The expression of the members of the panel of genes associated with hot flash symptoms can be either upregulated or downregulated. As shown above in TABLE 1, reference compounds can characteristically increase or decrease the expression of genes associated with hot flash symptoms. Thus, the nature of the regulation of expression, e.g., upregulation or downregulation, of genes associated with hot flash symptoms can also be used to determine the effect of a candidate compound on hot flash symptoms. For example, contacting a cell that expresses the estrogen receptor with estradiol results in the upregulation of BAD2 and HKII, while contact of the cell with 4-hydroxy tamoxifen results in the downregulation of these genes.

[0094] Accordingly, in certain embodiments, expression of at least one member of the panel of genes associated with hot flash symptoms can be upregulated in a cell that expresses the estrogen receptor or estrogen related receptor following contact with a candidate compound. In certain embodiments, expression of at least one member of the panel of genes associated with hot flash symptoms can be not upregulated in a cell that expresses the estrogen receptor or estrogen related receptor following contact with a candidate compound. In certain embodiments, expression of at least one member of the panel of genes associated with hot flash symptoms can be downregulated in a cell that expresses the estrogen receptor or estrogen related receptor following contact with a candidate compound. In certain embodiments, expression of at least one member of the panel of genes associated with hot flash symptoms can be not downregulated in a cell that expresses the estrogen receptor or estrogen related receptor following contact with a candidate compound.

[0095] In certain embodiments, expression of Type II Hexokinase, Multi Drug Resistance Gene, Parvalbumin, BAD2, Prolactin, Interleukin-18, Calpain, or EST196325 can be upregulated in a cell that expresses the estrogen receptor or estrogen related receptor following contact with the candidate compound. In certain embodiments, expression of Annexin 1, N27, or HBP1 can be not upregulated in a cell that expresses the estrogen receptor or estrogen related receptor following contact with the candidate compound. In certain embodiments, expression of Protein Kinase C subspecies epsilon can be not downregulated in a cell that expresses the estrogen receptor or estrogen related receptor following contact with the candidate compound.

[0096] In other embodiments, expression of Type II Hexokinase, Multi Drug Resistance Gene, Parvalbumin, BAD2, Prolactin, Interleukin-18, Calpain, or EST196325 can be not upregulated in a cell that expresses the estrogen receptor or estrogen related receptor following contact with the candidate compound. In certain embodiments, expression of Annexin 1, N27, or HBP1 can be upregulated in a cell that expresses the estrogen receptor or estrogen related receptor following contact with the candidate compound. In certain embodiments, expression of Protein Kinase C subspecies epsilon can be downregulated in a cell that expresses the estrogen receptor or estrogen related receptor following contact with the candidate compound.

[0097] In a preferred embodiment, expression of Type II Hexokinase, Multi Drug Resistance Gene, Parvalbumin, BAD2, Prolactin, Interleukin-18, Calpain, and EST196325 are upregulated in a cell that expresses the estrogen receptor or estrogen related receptor following contact with the candidate compound; expression of Annexin 1, N27, and HBP1 is not upregulated in a cell that expresses the estrogen receptor or estrogen related receptor following contact with the candidate compound; and expression of Protein Kinase C subspecies epsilon is not downregulated in a cell that expresses the estrogen receptor or estrogen related receptor following contact with the candidate compound.

[0098] The amount of the change in expression of a panel of genes associated with hot flash symptoms in a cell following contact with a candidate compound can also be assayed in order to determine the effects of the candidate compound on hot flash symptoms. A greater change in expression of genes associated with hot flash symptoms correlates with a more potent effect on hot flash symptoms. Further, the direction of regulation, either up or down, can be compared to the effect of the compounds with a known effect on hot flash symptoms.

[0099] For example, estradiol is the most potent therapeutic agent known to decrease the incidence of hot flash symptoms. If a candidate compound causes the expression of a gene that is upregulated following contact with estradiol, for example, HKII, to increase a similar or greater degree as estradiol, then the candidate compound can be determined to decrease the incidence of hot flash symptoms. The same relationship applies to the effects of tibolone, raloxifene, and 4-hydroxy tamoxifen on regulation of expression of genes associated with hot flash symptoms and the determined effect of a candidate compound on hot flash

symptoms. However, even if a candidate compound causes the expression of a gene, for example, HKII, to increase, but to a lesser degree than estradiol, the candidate compound may still decrease the incidence of hot flash symptoms.

[0100] In such situations, the relative amount of increase or decrease in expression of a gene associated with hot flash symptoms following contact with a candidate compound can be determined. For example, a candidate compound may cause the expression of a gene, for example HKII, to increase about two-fold, i.e., by about 100%. Notwithstanding the relative increase or decrease in expression of a given gene following contact with estradiol or tibolone, a candidate compound that results in about a two-fold increase in expression of a gene that is upregulated following contact with estradiol or tibolone is likely to decrease the incidence of hot flash symptoms.

[0101] Similarly, a candidate compound that results in an about two-fold decrease, i.e., by about 50%, in expression of a gene that is downregulated following contact with estradiol or tibolone is likely to decrease the incidence of hot flash symptoms. The converse is also true; a candidate compound that results in an about two-fold increase, i.e., by about 100%, in expression of a gene that is upregulated following contact with raloxifene or 4-hydroxy tamoxifen is likely to increase the incidence of hot flash symptoms. Similarly, a candidate compound that results in an about two-fold decrease, i.e., by about 50%, in expression of a gene that is downregulated following contact with raloxifene or 4-hydroxy tamoxifen is likely to increase the incidence of hot flash symptoms.

6.2.3. Determining the Effect of a Candidate Compound on Hot Flash Symptoms

[0102] The present invention provides methods of determining the effect of a candidate compound on hot flash symptoms based, in part, on the discovery of a correlation between regulation of a panel of genes associated with hot flash symptoms in a cell and contact with the cell by a reference compound with a known effect on hot flash symptoms. The genes associated with hot flash symptoms, reference compounds with known effects on hot flash symptoms, and the effects of such compounds on regulation of such genes are described extensively above. The effects of candidate compounds on hot flash symptoms can be determined by observing the effects of such compounds on the regulation of a panel of genes associated with hot flash symptoms.

[0103] Generally, the methods of the invention provide for exposing a cell that expresses the estrogen receptor or estrogen related receptor to a candidate compound, and determining the effect of the candidate compound on the regulation of one or more genes that are associated with hot flash symptoms. Preferably, the effects of the candidate compound on regulation of more than two, four, five, six, or eight genes that are associated with hot flash symptoms are determined. Most preferably, the effects of the candidate compound on regulation of about ten genes that are associated with hot flash symptoms are determined.

[0104] In certain embodiments, the effect of a candidate compound on the regulation of genes associated with hot flash symptoms can correlate with the effect of a compound that is selected from the group of estradiol, tibolone, raloxifene, and 4-hydroxy tamoxifen. In other embodiments, the effect of a candidate compound on the regulation of genes associated with hot flash symptoms can correlate with the effect of a compound that is selected from the group of estradiol and tibolone. In still other embodiments, the effect of a candidate compound on the regulation of genes associated with hot flash symptoms can correlate with the effect of a compound that is selected from the group of raloxifene and 4-hydroxy tamoxifen. In yet other embodiments, the effect of a candidate compound on the regulation of genes associated with hot flash symptoms can correlate with the effect of estradiol. In still other embodiments, the effect of a candidate compound on the regulation of genes associated with hot flash symptoms can correlate with the effect of tibolone. In yet other embodiments, the effect of a candidate compound on the regulation of genes associated with hot flash symptoms can correlate with the effect of raloxifene. In still other embodiments, the effect of a candidate compound on the regulation of genes associated with hot flash symptoms can correlate with the effect of 4-hydroxy tamoxifen.

[0105] In certain embodiments, the effect of the candidate compound on the regulation of a panel genes associated with hot flash symptoms can be different from the effect of a compound that is selected from the group consisting of estradiol, tibolone, raloxifene, and 4-hydroxy tamoxifen. Nonetheless, one of skill in the art can still determine the effect of the candidate compound on hot flash symptoms based upon the degree of similarity of effect on hot flash symptoms of the candidate compound to the effects of the reference compounds.

[0106] In general, the effect of the candidate compound on regulation of genes associated with hot flash symptoms, particularly the direction of regulation, can be compared to the effects of the reference compounds on regulation of the same genes. If the candidate compound regulates the expression of at least about half of the genes associated with hot flash symptoms that are tested similar to the manner in which estradiol or tibolone regulate the same genes, but no such genes are regulated similar to the manner in which raloxifene or 4-hydroxy tamoxifen regulate the same genes, the candidate compound can be determined to decrease the incidence of hot flash symptoms. If the candidate compound regulates the expression of at least about half of genes associated with hot flash symptoms that are tested similar to the manner in which raloxifene or 4-hydroxy tamoxifen regulate the same genes, but no such genes are regulated similar to the manner in which estradiol or tibolone regulate the same genes, the candidate compound can be determined to increase the incidence of hot flash symptoms. Preferably, expression of at least ten such genes is tested in determining the effect of the candidate compound, as shown in the following examples. However, as few as five genes associated with hot flash symptoms can be tested in determining the effect of a candidate compound hot flash symptoms.

[0107] For example, a candidate compound could increase the expression of at least about five genes associated with hot flash symptoms that are upregulated in a cell following contact with estradiol and/or tibolone, but not increase the expression of any genes that are upregulated in a cell following contact with raloxifene and/or 4-hydroxy tamoxifen. Such a candidate compound would be determined to decrease the incidence of hot flash symptoms. In another example, a candidate compound that can decrease the

expression of at least about five genes associated with hot flash symptoms that are downregulated in a cell following contact with estradiol and/or tibolone, but not increase the expression of any genes that are upregulated in a cell following contact with raloxifene and/or 4-hydroxy tamoxifen would also be determined to decrease the incidence of hot flash symptoms. Other examples of candidate compounds that would be determined to decrease the incidence of hot flash symptoms include those that increase the expression of at least about five genes associated with hot flash symptoms that are upregulated in a cell following contact with estradiol and/or tibolone, but not decrease the expression of any genes that are downregulated in a cell following contact with raloxifene and/or 4-hydroxy tamoxifen and those that can decrease the expression of at least about five genes associated with hot flash symptoms that are downregulated in a cell following contact with estradiol and/or tibolone, but not decrease the expression of any genes that are downregulated in a cell following contact with raloxifene and/or 4-hydroxy tamoxifen.

[0108] In another example, a compound that can increase the expression of at least about five genes that are upregulated in a cell following contact with raloxifene or 4-hydroxy tamoxifen but does not increase the expression of any genes that are upregulated in a cell following contact with estradiol or tibolone would be determined to increase the incidence of hot flash symptoms. In still another example, candidate compounds that decrease the expression of at least about five genes that are downregulated in a cell following contact with raloxifene and 4-hydroxy tamoxifen but do not decrease the expression of any genes that are downregulated in a cell following contact with estradiol or tibolone would be determined to increase the incidence of hot flash symptoms. Other examples of candidate compounds that would be determined to increase the incidence of hot flash symptoms include those that that can increase the expression of at least about five genes that are upregulated in a cell following contact with raloxifene or 4-hydroxy tamoxifen but does not decrease the expression of any genes that are downregulated in a cell following contact with estradiol or tibolone and those that decrease the expression of at least about five genes that are downregulated in a cell following contact with raloxifene and 4-hydroxy tamoxifen but do not increase the expression of any genes that are upregulated in a cell following contact with estradiol or tibolone.

[0109] In certain embodiments, the effect of the candidate compound on regulation of genes associated with hot flash symptoms can be compared to a reference expression profile of the panel of genes associated with hot flash symptoms. The reference expression profile of the panel of genes associated with hot flash symptoms is generally determined by contacting a cell with a compound selected from the group consisting of estradiol, tibolone, raloxifene, and 4-hydroxy tamoxifen. The reference pattern can be predetermined, i.e., known at the time the candidate compound is contacted to the cell to determine the effect of the candidate compound on regulation of hot flash symptoms. The reference pattern can comprise any set of one or more genes and effects on hot flash symptoms listed in TABLE 1.

[0110] In other embodiments, the methods further comprise comparing a cell's expression of the panel of genes associated with hot flash symptoms following contact with a candidate compound with a cell's expression of the panel of genes associated with hot flash symptoms following contact with a compound that has a known effect on hot flash symptoms. The compound that has a known effect on hot flash symptoms can be any such compound known to one of skill in the art without limitations. In certain embodiments, the compound that has a known effect on hot flash symptoms can be selected from the group that consists of estradiol, tibolone, raloxifene, and 4-hydroxy tamoxifen. In such embodiments, the compound or compounds with known effects on hot flash symptoms are contacted with a cell in parallel with one or more candidate compounds as a control assay. The effects of the candidate compounds and reference compounds on regulation of expression of genes associated with hot flash symptoms can be compared to determine the effect of the candidate compound on hot flash symptoms.

6.2.4. Cells and Cell Lines that Express the Estrogen Receptor and Estrogen Related Receptor

[0111] The present invention is based, in part, on assessing the regulation of genes associated with hot flash symptoms in cells that express the estrogen receptor or estrogen related receptor following contact with compounds that can modulate the estrogen receptor or estrogen related receptor. Accordingly, certain aspects of the invention rely on contacting a cell that expresses the estrogen receptor or estrogen related receptor with a candidate compound.

[0112] Any cell that is known by one of skill in the art to express the estrogen receptor or estrogen related receptor, without limitation, may be used in the methods of the invention. In certain embodiments, the cell can express the estrogen receptor. In other embodiments, the cell can express the estrogen related receptor. Such cells that express one of these receptors are generally mammalian cells. In certain embodiments, the mammalian cell can be selected from the group that consists of a rat cell, a mouse cell, a monkey cell, a chimpanzee cell, and a human cell.

[0113] Further, the cell can be derived from any organ or tissue that expresses the estrogen receptor or the estrogen related receptor. For example, in certain embodiments, the cell that expresses the estrogen receptor can be a pituitary cell. In other embodiments, the cell that expresses the estrogen receptor can be a hypothalamus cell. In still other embodiments, the cell that expresses the estrogen related receptor can be any central or peripheral nervous system cell that expresses the estrogen related receptor.

[0114] The cell that expresses the estrogen receptor or estrogen related receptor and that is used in the methods of the invention is preferably a cell suitable for propagation in cell culture for an indefinite period of time. Any such cell that is known by one of skill in the art to express the estrogen receptor or estrogen related receptor and to be suitable for propagation in cell culture for an indefinite period of time can be used in the methods of the invention, without limitation. In certain embodiments, the cell that expresses the estrogen receptor can be selected from the group of a GH3 cell, a GH4 cell, a PR1 cell, a MtT/E-2 cell, a alphaT3-1 cell, a D12 cell, an RCF-8 cell, and a GT1-7 cell. In a preferred embodiment, the cell that expresses the estrogen receptor is a GH3 cell. In certain embodiments, the cell that expresses the estrogen related receptor can be selected from the group that consists of an A172 glioma cell, a MCF10a cell, a MCF12 cell, a MDA-MB-231 cell, a

MDA-MB-435 cell, a MDA-MB-436 cell, a MDA-MB-468 cell, a Hs 578T cell, a BT 20 cell, a BT 474 cell, a BT 549 cell, a SKBr 3 cell, a ZR 75.1 cell, a T47D cell, and a MCF7 cell.

[0115] Further, the cell that expresses the estrogen receptor can express any estrogen receptor known by one of skill in the art without limitation. In certain embodiments, the estrogen receptor that is expressed by the cell can be estrogen receptor α. In other embodiments, the estrogen receptor that is expressed by the cell can be estrogen receptor β. In still other embodiments, the cell that expresses the estrogen receptor can express both estrogen receptor a and estrogen receptor β.

[0116] Similarly, the cell that expresses the estrogen related receptor can express any estrogen related receptor known to one of skill in the art without limitation. In certain embodiments, the cell that expresses the estrogen related receptor can express estrogen related receptor α. In other embodiments, the cell that expresses the estrogen related receptor can express estrogen related receptor β. In yet other embodiments, the cell that expresses the estrogen related receptor can express estrogen related receptor γ. In still other embodiments, the cell that expresses the estrogen related receptor can express two or three estrogen related receptors, each of which is selected from the group that consists of estrogen related receptor α, estrogen related receptor β, and estrogen related receptor γ.

6.2.5. High Throughput Methods for Ouantifying the Expression of Genes Associated with Hot Flash Symptoms

[0117] In certain aspects, the present invention provides high throughput methods for determining the effect of a plurality of candidate compounds on hot flash symptoms. High throughput methods for determining the effect of a plurality of compounds on hot flash symptoms generally comprise separately exposing samples of cells that express the estrogen receptor or estrogen related receptor to each member of the plurality of candidate compounds. The effects of each of the plurality of candidate compounds on the regulation of expression of a panel of genes associated with hot flash symptoms can then be determined for each of the samples of cells. Using the above-described correlation between regulation of expression of such genes and the effect of a reference compound on hot flash symptoms, the effects of the plurality of compounds can thus be identified.

[0118] High throughput methods generally rely on simultaneously determining the effects of a large number of compounds on the regulation of expression of a panel of genes associated with hot flash symptoms in a sample of cells. The high throughput methods used in connection with the invention are further described with reference to particular embodiments of such high throughput methods as described below. Further guidance regarding these particular embodiments of the high throughput methods for determining the effect of candidate compounds on hot flash symptoms may be found in U.S. Pat. No. 6,238,869, which is incorporated by reference in its entirety. This patent describes generic methods that can be readily be adapted to determine the effect of candidate compound on regulation of expression of genes associated with hot flash symptoms as described below. However, any high throughput method known to one of skill in the art to be useful in the determination of the effects of a large number of compounds on regulation of gene expression, without limitation, can be used in the methods of the invention.

[0119] Briefly, the preferred high throughput method comprises separately exposing samples of cells to each member of a plurality of candidate compounds. The samples of cells are then lysed. The lysates are exposed to a plurality of gene-specific nuclease protection probes, each of which can hybridize to a member of a panel of genes associated with hot flash symptoms, as described above. The lysates are then contacted with a nuclease with single-stranded nuclease activity, such as, for example, S1 nuclease. Following digestion to completion with this nuclease, the remaining RNA in the lysates can optionally be degraded.

[0120] The remainder of the method comprises detecting the gene specific nuclease protection probes or RNA protected from degradation by the gene-specific nuclease protection probes. In one embodiment, the lysates are contacted with a surface containing regions adapted to specifically associate with each of the gene-specific nuclease protection probes. The presence and amount of each gene-specific nuclease protection probe can be detected by contacting the gene-specific nuclease protection probes with a detection probe that specifically associates with the gene-specific nuclease protection probes. The detection probe can then be detected directly or indirectly.

[0121] In addition, certain other methods for quantifying the expression of genes associated with hot flash symptoms, as described in Section 5.2.6, below, can be adapted by one of skill in the art to identify the effects of a large number of candidate compounds and thus can be used as high throughput methods. Therefore, the high throughput methods for identifying the effects of candidate compounds on hot flash symptoms are not limited to the above-described embodiments, but also include those described below as well. Further, the methods for identifying genes associated with hot flash symptoms can also be adapted to determine the effects of candidate compounds on regulation of expression of genes associated with hot flash symptoms and thus can be used to determine the effects of such candidate compounds on hot flash symptoms.

6.2.6. Other Methods for Quantifying the Expression of Genes Associated with Hot Flash Symptoms

[0122] In certain aspects, the invention provides methods for determining the effect of a candidate compound on hot flash symptoms by determining the effect of the candidate compound on expression of a panel of genes associated with hot flash symptoms. Generally, the effect of the candidate compound on expression of genes associated with hot flash symptoms can be determined by quantifying the amount of mRNA expressed from a gene associated with hot flash symptoms by a cell in the presence and absence of the candidate compound. By comparing the amount of mRNA expressed from a gene associated with hot flash symptoms by a cell that has been exposed to a candidate compound to the amount of mRNA expressed from a gene associated with hot flash symptoms by a cell that has not been exposed to the candidate compound, the effect of the candidate compound on expression of genes associated with hot flash symptoms can be determined.

[0123] Any method known by one of skill in the art to be useful in detecting the presence and amount of an mRNA expressed from a gene associated with hot flash symptoms can be used in the methods of the invention, without limitation. For example, the presence and amount of an mRNA expressed from a gene associated with hot flash symptoms can be detected with a technique that is selected from the group consisting of reverse transcription real time PCR, quantitative reverse transcription PCR, Northern blot assays, dot blot assays, reverse dot blot assays, S1 nuclease protection assays, primer extension, RNAse protection assays, 5'-nuclease assays, reporter gene assays, branched DNA assays, bead array assays, and multiplexed array mRNA assays. In a preferred embodiment, the presence and amount of an mRNA expressed from a gene associated with hot flash symptoms is detected with a multiplexed array mRNA assay, as described above.

[0124] Many of the above referenced techniques, as well as protocols for performing them, are described in Ausubel et al., eds., *Current Protocols in Molecular Biology,* 2002, Vol. 4, Unit 4, Ch. 6-9 and Unit 15, Ch. 5, which is hereby incorporated by reference in its entirety. For example, Northern blot assays, S1 nuclease protection assays, primer extension, RNAse protection assays, and reverse transcription PCR are well known to in the art and are each described in detail in this reference. One of ordinary skill in the art can readily adapt these protocols for detecting the presence and amount of an mRNA that is expressed by genes associated with hot flash symptoms.

[0125] Further, the presence and amount of an mRNA expressed from genes associated with hot flash symptoms can be accomplished using a dot blot format. In the dot blot format, the unlabeled amplified sample is bound to a solid support, such as a membrane, the membrane incubated with labeled probe under suitable hybridization conditions, the unhybridized probe removed by washing, and the filter monitored for the presence of bound probe. When multiple samples are analyzed with a single probe, the dot blot format is quite useful. Many samples can be immobilized at discrete locations on a single membrane and hybridized simultaneously by immersing the membrane in a solution of probe.

[0126] An alternate method that is quite useful when large numbers of different probes are to be used is a "reverse" dot blot format, in which the amplified sequence contains a label, and the probe is bound to the solid support. This format can be especially useful if the assay methods of the present invention is used as one of a battery of methods to be performed simultaneously on a sample. In this format, the unlabeled probes specific for a gene or several genes associated with hot flash symptoms are bound to the membrane and exposed to the labeled sample under appropriately stringent hybridization conditions. Unhybridized labeled sample is then removed by washing under suitably stringent conditions, and the filter is then monitored for the presence of bound sequences.

[0127] Both the forward and reverse dot blot assays can be carried out conveniently in a microtiter plate; see U.S. patent application Ser. No. 695,072, filed May 3, 1991, which is a CIP of U.S. patent application Ser. No. 414,542, filed Sep. 29, 1989, now abandoned, each of which is incorporated herein by reference in its entirety. The probes can be attached to bovine serum albumen (BSA), for example, which adheres to the microtiter plate, thereby immobilizing the probe. Another example of a method of using one or more oligonucleotides specific for genes associated with hot flash symptoms to detect the presence and amount of mRNA expressed from such genes is described in U.S. Pat. No. 6,383,756, which provides a method for detecting a nucleic acid bound to a membrane, and which is hereby incorporated by reference in its entirety.

[0128] In addition, Bustin, 2002, *J. Mol. Endocrinol.* 29(1):23-39; Bustin, 2000, *J. Mol. Endocrinol.*25(2):169-93; and Freeman et al., 1999, *Biotechniques.* 26(1):112-22, 124-5; each of which are incorporated by reference in their entirety, review the state of the art of real time reverse transcription PCR and provide methods for performing such reactions. These references also describe quantitative reverse transcription PCR, which is further reviewed by Richards and Poch, 2002, *Mol. Biotechnol.* 21(1):19-37, which is hereby incorporated by reference in its entirety. These references teach reaction conditions and parameters that can easily be adapted by one of skill in the art for detecting the presence and amount of an mRNA expressed from genes that are associated with hot flash symptoms.

[0129] 5'-nuclease assays generally comprise contacting a primer hybridized to the nucleic acid to be detected with an enzyme with 5'-nuclease activity. The enzyme with 5'-nuclease activity then fragments a probe that is also hybridized to the nucleic acid to be detected in a 5'-nuclease reaction. The probe can be labeled with a detectable moiety that enables detection of fragmentation of the probe. Such methods are based on those described in U.S. Pat. Nos. 6,214,979, 5,804,375, 5,487,972 and 5,210,015, each of which is hereby incorporated by reference in its entirety. One of skill in the art is readily able to recognize suitable probes and primers for detecting a particular nucleic acid of known sequence for use in a 5'-nuclease assay for detecting the presence and amount of an mRNA expressed from genes associated with hot flash symptoms.

[0130] In another example, the presence and amount of mRNA expressed from genes associated with hot flash symptoms can be detected with a branched-DNA assay. In such methods, a dendrimer monomer is constructed of two DNA strands that share a region of sequence complementarity located in the central portion of each strand. When the two strands anneal to form the monomer the resulting structure has a central double-stranded center bordered by four single-stranded ends. A dendrimer can be assembled from monomers by hybridization of the single stranded ends of the monomers to each other, while still leaving many single-stranded ends free. These free single-stranded ends can have the sequences of any nucleic acid that can hybridize to an mRNA expressed from genes associated with hot flash symptoms. A dendrimer can be detectably labeled with any detectable moiety known to one of skill in the art without limitation, as described above in connection with the high throughput methods for quantifying an mRNA expressed from genes associated with hot flash symptoms.

[0131] Dendrimers can then be used as a probe, in, for example, the "dot blot" assays described above. In addition, a dendrimer can be used as a probe in any method known to one of skill in the art in which the probe is directly detected. A probe is directly detected when the presence of the probe can be determined without any subsequent reaction or

modification, such as a dot blot or Northern hybridization. Further guidance on the selection and use of dendrimers as probes to detect the presence and amount of an mRNA expressed from genes associated with hot flash symptoms may be found in U.S. Pat. Nos. 6,261,779 and in Nilsen et al., 1997, *J. Theoretical Biology* 187:273-284, Capaldi et al., 2000, *Nucleic. Acids Res.,* 28(7):21e, Wang et al., 1998, *J. Am. Chem. Soc.* 120:8281-8282, and Wang et al., 1998, *Electroanalysis* 10(8):553-556, each of which is hereby incorporated by reference in its entirety.

[0132] A reporter gene assay can also be used to detect the presence and amount of an mRNA expressed from genes associated with hot flash symptoms. Such assays generally rely on recombinantly linking the transcriptional control region of a gene associated with hot flash symptoms to the coding region of a reporter gene. The presence and amount of expression of the reporter gene can then be determined. The presence and amount of an mRNA expressed by genes associated with hot flash symptoms can then be extrapolated based upon the presence and amount of the reporter gene that is detected. Representative reporter gene assays that can easily be adapted by one of skill in the art for use in the methods of the invention are described by Storz et al., 1999, *Anal. Biochem.* 276:97-104 and Terstappen et al., 2000, *J. Biomol. Screen.* 5:255-262, each of which is incorporated by reference in its entirety.

[0133] The presence and amount of an mRNA expressed from genes associated with hot flash symptoms can also be determined using a bead array-based assay. In such assays, oligonucleotides specific for the mRNA to be detected are coupled to fluorescently-detectable beads. The mRNA is then reverse transcribed into labeled cDNA, and hybridized to the detectable beads. The presence and amount of the labeled cDNA can then be detected, revealing the presence and amount of the mRNA expressed from the gene associated with hot flash symptoms. One of ordinary skill in the art can readily adapt the protocols described in Yang et al., 2001, *Genome Res.* 11:1888-98 and in U.S. Pat. Nos. 6,562,569, 6,514,771, 6,468,811, 6,387,707, 6,376,256, 6,255,116, and 6,251,691, each of which is incorporated by reference in its entirety, to detect the presence and amount of an mRNA expressed from genes associated with hot flash symptoms.

[0134] Of course, the presence and amount of an mRNA expressed from genes associated with hot flash symptoms can be determined by reverse transcribing the mRNA into cDNA and detecting the presence and amount of cDNA product. Thus, any of the above-described methods suitable for the detection of DNA in addition to RNA can be adapted to detect cDNA derived from mRNA expressed from genes associated with hot flash symptoms.

[0135] Similarly, the presence and amount of mRNA expressed from genes associated with hot flash symptoms can also be determined by quantifying the amount of protein translated from the mRNA. As one of skill in the art is aware, the amount of protein translated from a given transcript, absent translational regulation, depends on the amount of mRNA transcript. Thus, the effects of candidate compounds on regulation of genes associated with hot flash symptoms can also be determined by detecting the presence and amount of proteins expressed from genes associated with hot flash symptoms.

[0136] The presence and amount of protein expressed from genes associated with hot flash symptoms can be determined by any suitable method known by one of skill in the art without limitation. For example, and not by way of limitation, the presence and amount of protein expressed from genes associated with hot flash symptoms can be determined using a western blot assay, an ELISA assay, a cytokine bead array, multiplexed protein detection assays, an immunofluorescence assay, and the like.

[0137] Many of the above-referenced techniques, as well as protocols for performing them, are described in Ausubel et al., eds., *Current Protocols in Molecular Biology,* 2002, Vol. 1, Unit 10, Ch. 3, and Vol. 2, Unit 11 Ch. 1-6., which is hereby incorporated by reference in its entirety. For example, western blot assays, ELISA assays, and immunofluorescence assays are well known to in the art and are each described in detail in this reference. One of ordinary skill in the art can readily adapt these protocols for detecting the presence and amount of protein that is expressed by genes associated with hot flash symptoms.

[0138] In addition, U.S. Pat. No. 6,576,478, which is incorporated by reference in its entirety, describes an array suitable for cytokine bead assays, multiplexed fluorescence assays, and other high throughput assays for determining the presence and amount of protein expressed from genes associated with hot flash symptoms. The device, as well as the device described in U.S. Pat. No. 6,558,960, also incorporated by reference in its entirety, can readily be adapted by one of skill in the art for use in such methods. U.S. Pat. Nos. 6,531,283 and 6,511,802, each of which is hereby incorporated by reference in its entirety, provide additional guidance regarding such assays for determining the presence and amount of protein expressed from genes associated with hot flash symptoms.

6.2.7. Primers and Probes Useful in Certain Methods for Quantifying the Expression of Genes Associated with Hot Flash Symptoms

[0139] The present invention further provides nucleic acid primers that are useful in certain methods for quantifying the expression of genes associated with hot flash symptoms. As one of skill in the art will readily recognize, certain of such methods described above rely on the use of oligonucleotide primers to amplify nucleic acids that comprise sequences of genes associated with hot flash symptoms. Methods of using such primers to reverse transcribe RNA and/or to amplify DNA of known sequence are well-known to the art and will not be presented in detail here. The primers, their sequences, and the genes from which sequences that the primers can amplify are derived are presented in TABLE 2, below.

TABLE 2

Gene	Primer 1 (Forward Primer)	Primer 2 (Reverse Primer)
HKII	M68971 211F: ATATGATCGCCTGCTTATTCA SEQ ID NO:28	M6897 1378B: AAAGGTAGGCAACATTTTCAC SEQ ID NO:29
HKII	M68971 577F: TTGACCACATCGCCGAATGC SEQ ID NO:30	M68971 893B: AGTGCCCACAATGAGACCAATC SEQ ID NO:31
Parvalbumin	AI175539 250F: CCCGTCCTTGTCTCCAG SEQ ID NO:32	AI175539 402B: AGAAAAAGAGTGCGGATGATG SEQ ID NO:33
Parvalbumin	AI175539 41F: AGCATTTTCCAGAAGAGTGGTGTC SEQ ID NO:34	AI175539 285B: ACAAAGACGCTGATGGCTGC SEQ ID NO:35
Activin beta E	AF089825 669F: GAGCACCAAACCACTTCCTC SEQ ID NO:36	AF089825 918B: CTACAACATAAGGGGGTCTC SEQ ID NO:37
Activin beta E	AF089825 492F: AAATCCACTTCAACCTACCGCTC SEQ ID NO:38	AF089825 941B: TCGTCTACAACATAAGGGGGTCTC SEQ ID NO:39
Glutamate receptor	U08255 642F: TCTATGACAGCGAGTATGATA SEQ ID NO:40	U08525 945B: CAAGGGCACTGTGGACCAGAT SEQ ID NO:41
Glutamate receptor	U08255 1030F: TAACCACCGCATCTCTTCCCTG SEQ ID NO:42	U08255 1336B: TGTGCCAAGGATTTCAAACTGG SEQ ID NO:43
PKC epsilon	M18331 512F: CAGAATGGGAGCCGTCACTTC SEQ ID NO:44	M18331 836B: AGCGCACTTCGTAATAATGAG SEQ ID NO:45
PKC epsilon	M18331 1298F: TTTGACAACCGAGGAGAGGAGC SEQ ID NO:46	M18331 1631B: CCTTGGTCTGGAAGCAGCAATAG SEQ ID NO:47
EST 203549	AI009098 115F: GGGGAACTGTGTAGGACCTT SEQ ID NO:48	AI009098 395B: ATGTAAAAATGCCACCTCACT SEQ ID NO:49
EST 203549	AI009098 61F: TTCAAACCTGTCCAACCAGCC SEQ ID NO:50	AI009098 382B: TTGTGGGTAAAGAAAGAGGGGTC SEQ ID NO:51
VAP1	AF034582 1239F: TCGCATCCGTGTCTACTCCATC SEQ ID NO:52	AF034582 1573B: GGAAGTCCTTTTCTGTCACCACC SEQ ID NO:53
VAP1	AF034582 313F: CAGAACCACCCCATTTACCTG SEQ ID NO:54	AF034582 655B: TGTTTACATCCAAGGCTCTCACTG SEQ ID NO:55
MDR	M81855 2147F: CGAAAGAGGATGTGGATGAAGATG SEQ ID NO:56	M81855 2468B: ATGTATCGGAGTCGCTTGGTGAGG SEQ ID NO:57
MDR	M81855 2153F: AGGATGTGGATGAAGATGTGCC SEQ ID NO:58	M81855 2468B: ATGTATCGGAGTCGCTTGGTGAGG SEQ ID NO:59
Arginino-succinate synthetase	X12459 448F: GGAGGATGCCCGAGTTTTACAAC SEQ ID NO:60	X12459 766B: AAGAGGTCCAAGGATGTGCTGTGG SEQ ID NO:61
Arginino-succinate synthetase	X12459 730F: AAGATGGCACTACCCACAGCAC SEQ ID NO:62	X12459 1057B: TTTCCTTCCACCCGTTCCTG SEQ ID NO:63

TABLE 2-continued

Gene	Primer 1 (Forward Primer)	Primer 2 (Reverse Primer)
BAD2	U02553 865F: ATCAAGGATGCTGGAGGAAGGG SEQ ID NO:64	U02553 1193B: TAGTTCAGGGCACTGTTCGTGG SEQ ID NO:65
BAD2	U02553 717F: CTTGGGTATCACTGCTTTGA SEQ ID NO:66	U02553 990B: ATAATACTCCGCCTCTGCTTC SEQ ID NO:67
Prolactin	AF022935 145F: TGTTCTGGTGGCGACTGCCAGACACCT SEQ ID NO:68	AF022935 528B: TATCTTTTCAATCCCTTCAAGAAGCCG SEQ ID NO:69
2-alpha-1 globin	X56325 299F AAGAACTGCTGGGGGAAGATTG SEQ ID NO:70	X56325 562B: TTGCCGTGAGCCTTGACCTG SEQ ID NO:71
RNR1	L08595 305F: ACAACTACAGCACAGGCTACGACG SEQ ID NO:72	640B: AGAAGAGTGAAAGGCGGGAGAC SEQ ID NO:73
RNR1	L08595 541F: GACGATCCGGGCTCCCTTCAC SEQ ID NO:74	L08595 780B: ATGGATGCCGGCTTGCGAATG SEQ ID NO:75
IL18	AY077842 334F: GACCACTTTGGCAGACTTCACTG SEQ ID NO:76	AY077842 546B: CCTTCCATCCTTCACAGATAGGG SEQ ID NO:77
IL18	AY077842 435F: GCCTGATATCGACCGAACAGC SEQ ID NO:78	AY077842 709B: ATCATCTTCCTTTTGGCAAGC SEQ ID NO:79
ARL gene 4	X77235 135F: ACTTCCATCCTATCCAGCCTGC SEQ ID NO:80	X77235 410B: CACCACAAACACAATGCCATCTG SEQ ID NO:81
ARL gene 4	X77235 301F: GCAATTCCAAAACAGTCAC SEQ ID NO:82	X77235 617B: TTTAGCCCATCTCCTATGATT SEQ ID NO:83
Calpain	Calpain 1424F: AGGCTACGCTGTCTACCAGATTCC SEQ ID NO:84	Calpain 1791B: AACACCCTCAAGCAGAAGTCACC SEQ ID NO:85
Calpain	Calpain 1224F: TGGACACGGGGTTCTACA SEQ ID NO:86	Calpain 1356B: CAACTCCTTGGGAATCTGGTA SEQ ID NO:87
EST 196325	EST196325 131F: GGAGCCATTGTTCACATTACCG SEQ ID NO:88	EST196325 356B: TACCCTGCCTTCTTCTCTCTGGAG SEQ ID NO:89
EST 196325	EST196325 150F: CCGACCAGCAACACAGAGC SEQ ID NO:90	EST196325 431B: TTCGCCGTAAAACATCAGCAT SEQ ID NO:91
CPP32	CPP32 362F: GGAGCAGTTTTGTGTGTGTGATTC SEQ ID NO:92	CPP32 630B: TGCGGTAGAGTAAGCATACAGGAAG SEQ ID NO:93
CPP32	CPP32 492F: GCCGAAACTCTTCATCATTCA SEQ ID NO:94	CPP32 796B: GATCTGTTTCTTTGCGTGGAA SEQ ID NO:95
Annexin1	NM_012904 822F: TGGAACTGAAGGGTGACATTGAG SEQ ID NO:96	NM_012904 1041B: ATGGCTTGGCAGAGAGGGATTC SEQ ID NO:97
Annexin1	NM_012904 824F: GAACTGAAGGGTGACATTGAG SEQ ID NO:98	NM_012904 1089B: GGGATGTTTAGTTTCCTCCAC SEQ ID NO:99

TABLE 2-continued

Gene	Primer 1 (Forward Primer)	Primer 2 (Reverse Primer)
EST AI014169	AI014169 212F: CTGACACAGGACACGGAACAAAG SEQ ID NO:100	AI014169 393B: GGTGACACTCTTACATTGAGATGCC SEQ ID NO:101
EST AI014169	AI014169 171F: CACAGTTCTCGGGTGGAGT SEQ ID NO:102	AI014169 360B: CATTGAGATGCCCTAACAGTG SEQ ID NO:103
HBP1	U09551 540F: GACCACTGGAAGGAAGAAACACC SEQ ID NO:104	U09551 864B: CAGACTCACCGAATGACACACTCTC SEQ ID NO:105
HBP1	U09551 840F: GAGAGTGTGTCATTCGGTGAGTCTG SEQ ID NO:106	U09551 1131B: CGGAAGAGTCCATAGGTGTGAAGTC SEQ ID NO:107
Amiloride Binding Protein	X73911 819F: TGGCTCGGAAATACGCAGTTG SEQ ID NO:108	X73911 1150B: AGGTGTGTGTCCTCCATACAGTGC SEQ ID NO:109
Amiloride Binding Protein	X73911 1367F: GGTGGCTTCAACTTCTATGCGG SEQ ID NO:110	X73911 1687B: CCAGGGATTGGTGAGGTTTTCC SEQ ID NO:111

[0140] Furthermore, the present invention provides probes that can also be used in certain methods for quantifying the expression of genes associated with hot flash symptoms. As one of skill in the art will readily recognize, certain of such methods described above rely on the use of nucleic acid probes to detect and quantify nucleic acids that comprise sequences of genes associated with hot flash symptoms. Methods of using such probes to detect and quantify nucleic acids of known sequence in, for example, RNAse protection assays, dot blots, and the like, are well-known to the art and will not be presented in detail here. The regions of genes associated with hot flash symptoms that can be used as probes to detect and quantify nucleic acids from such genes are presented in TABLE 3, below.

TABLE 3

Gene Name	Sequence	Length of cDNA
MDR	2147–2468	321 bp
Parvalbumin	41–285	244 bp
HKII	577–893	316 bp
BAD2	865–1193	294 bp
PKCϵ	1298–1631	333 bp
IL18	334–546	213 bp
Calpain	1224–1356	234 bp
EST196325	131–356	226 bp
Annexin1	822–1041	220 bp
EST207724(N27)	212–393	180 bp
HBP1	840–1131	292 bp
PRL	145–528	383 bp

6.3. Methods of Identifying Candidate Compounds that Decrease the Incidence of Hot Flash Symptoms

[0141] In another aspect, the invention provides methods of identifying candidate compounds that decrease the incidence of hot flash symptoms. These methods generally comprise performing a method of the invention for determining the effect of a candidate compound on hot flash symptoms, and additionally determining that the candidate compound decreases the incidence of hot flash symptoms.

[0142] A candidate compound can be determined to decrease the incidence of hot flash symptoms based upon the effect of the candidate compound on regulation of genes associated with hot flash symptoms as extensively described above. In certain embodiments, the effect of the candidate compound on regulation of expression of genes associated with hot flash symptoms can be compared to a reference pattern of expression, as described above. In other embodiments, the effect of the candidate compound on regulation of expression of genes associated with hot flash symptoms can be compared to the effect of a compound with a known effect on regulation of expression of genes associated with hot flash symptoms, as described above.

6.4. Kits for Determining the Effect of a Candidate Compound on Hot Flash Symptoms

[0143] In another aspect, the invention provides kits for determining the effect of a compound on hot flash symptoms. The kits generally comprise at least one primer or probe that can be used to detect the presence and amount of an expression product of a member of a panel of genes associated with hot flash symptoms. The primer or probe can be any primer or probe known by one of skill in the art to be useful for detecting the presence and amount of an expression product of a member of a panel of genes associated with hot flash symptoms without limitation. The members of the panel of genes can be any genes associated with hot flash symptoms known by one of skill in the art without limitation, especially those described above.

[0144] In certain embodiments, the primer or probe can be used to detect an mRNA expressed from a member of a panel of genes associated with hot flash symptoms. Primers and probes suitable for such kits are extensively described in Section 5.2.7., above. In other embodiments, the primer or

probe can be used to detect a protein expressed from a member of a panel of genes associated with hot flash symptoms.

[0145] In another aspect, the invention provides kits for determining the effect of one or more candidate compounds on hot flash symptoms. In certain embodiments, the kit comprises at least one gene-specific nuclease protection probe that is specific for a member of a panel of genes associated with hot flash symptoms; a surface having multiple spatially discrete regions, wherein at least two of the regions are substantially identical and wherein each region comprises at least two different oligonucleotide anchors; at least one bifunctional linker, wherein each bifunctional linker comprises a first region that can specifically bind to one of the oligonucleotide anchors and a second region that specifically binds to one of the gene-specific nuclease protection probe(s); and at least one detection probe, wherein the detection probe(s) specifically binds to the gene-specific nuclease protection probe(s).

[0146] In another embodiment, the kit for determining the effect of a compound on hot flash symptoms, comprises at least one gene-specific nuclease protection probe that is specific for a member of a panel of genes associated with hot flash symptoms and that can be directly or indirectly detectable; and a surface having multiple spatially discrete regions, at least two of which regions are substantially identical, and wherein the regions are adapted to specifically bind to the gene-specific protection probe(s).

[0147] The kits of the invention can comprise any components described as useful in the methods of the invention, above, without limitation. For example, gene-specific nuclease protection probes, detection probes, primers, and probes described as useful in the methods of the invention may also be included in a kit of the invention.

[0148] In certain embodiments, a kit of the invention can comprise instructions for determining the effect of a compound on hot flash symptoms. In certain embodiments, the kit can comprise instructions for identifying a compound that decreases the incidence of hot flash symptoms.

6.5. Compositions for Determining the Effect of a Candidate Compound on Hot Flash Symptoms

[0149] In yet another aspect, the invention provides compositions suitable for determining the effect of a compound on hot flash symptoms. The compositions may also be used in the methods and kits of the invention. In certain embodiments, the composition comprises a plurality of gene-specific nuclease protection probes, wherein each member of the plurality of gene-specific nuclease protection probes hybridizes under stringent conditions to an mRNA expressed from a member of a panel of genes associated with hot flash symptoms. The member of the panel of genes can be any member of a panel of genes associated with hot flash symptoms known by one of skill in the art without limitation.

[0150] In other embodiments, the composition comprises one or more primers that can be used to determine the presence and amount of an expression product of one or more genes associated with hot flash symptoms and a suitable buffer, diluent, or excipient. In certain embodiments, the primer or probe can be used to detect an mRNA expressed from a member of a panel of genes associated with hot flash symptoms. Primers and probes suitable for such kits are extensively described in Section 5.2.7., above. In other embodiments, the primer or probe can be used to detect a protein expressed from a member of a panel of genes associated with hot flash symptoms.

[0151] In certain embodiments, the member of the panel of genes associated with hot flash symptoms can be selected from the group consisting of Activin Beta E, Type II Hexokinase, Multi Drug Resistance Gene, Parvalbumin, BAD2, Prolactin, Argininosuccinate Synthetase, Ribonucleoside Reductase 1, Interleukin-18, ARL gene 4, Calpain, EST196325, CPP32, EST208064, 2-alpha-1 globin, Amiloride Binding Protein, Annexin 1, N27, HBP1, D-binding protein, FE65, Protein Kinase C type I, Glutamate Receptor subunit d1, VAP1, Protein Kinase C subspecies epsilon, EST203549, and Heat Shock Transcription Factor 1. In a preferred embodiment, the member of the panel of genes is selected from the group consisting of Type II Hexokinase, Multi Drug Resistance Gene, Parvalbumin, BAD2, Prolactin, Interleukin-18, Calpain, EST196325, Annexin 1, N27, HBP1, and Protein Kinase C subspecies epsilon.

6.6. Arrays for Determining the Effect of a Candidate Compound on Hot Flash Symptoms

[0152] In yet another aspect, the invention provides arrays useful for the identification of the effect of a plurality of compounds on hot flash symptoms. In certain embodiments, the array can comprise a non-porous surface; and a plurality of different oligonucleotides connected with the surface, wherein at least one of the oligonucleotides hybridizes under stringent conditions to a member of a panel of genes associated with hot flash symptoms, and wherein each of the different oligonucleotides is connected with the surface in a different predetermined region of the surface. The member of the panel of genes can be any member of a panel of genes associated with hot flash symptoms known by one of skill in the art without limitation.

[0153] In certain embodiments, the member of the panel of genes can be selected from the group consisting of Activin Beta E, Type II Hexokinase, Multi Drug Resistance Gene, Parvalbumin, BAD2, Prolactin, Argininosuccinate Synthetase, Ribonucleoside Reductase 1, Interleukin-18, ARL gene 4, Calpain, EST196325, CPP32, EST208064, 2-alpha-1 globin, Amiloride Binding Protein, Annexin 1, N27, HBP1, D-binding protein, FE65, Protein Kinase C type I, Glutamate Receptor subunit d1, VAP1, Protein Kinase C subspecies epsilon, EST203549, and Heat Shock Transcription Factor 1. In a preferred embodiment, the member of the panel of genes is selected from the group consisting of Type II Hexokinase, Multi Drug Resistance Gene, Parvalbumin, BAD2, Prolactin, Interleukin-18, Calpain, EST196325, Annexin 1, N27, HBP1, and Protein Kinase C subspecies epsilon.

7. EXAMPLES

[0154] The invention is described in reference to a number of examples presented below. The examples are intended to provide illustration of certain embodiments of the invention, and should not be construed to limit the invention in any way.

7.1. Example 1

Generation of Reference Expression Profiles of a Panel of Genes Associated with Hot Flash Symptoms

[0155] The following example describes the generation of expression profiles of a panel of genes whose expression level is associated with the increase or decrease of hot flash symptoms using the compounds estradiol, raloxifene, tibolone, and 4-hydroxy tamoxifen. Estradiol and raloxifene are known to have a decreasing effect on hot flash symptoms, while tibolone and 4-hydroxy tamoxifen are known to have an increasing effect on hot flash symptoms.

[0156] Treatment of GH3 Cells and Preparation of Cell Lysates. In 96-well V-bottom dishes, 50,000 GH3 cells per well were plated in 200 μl phenol red free Ham's F-12K media supplemented with 2.5% charcoal stripped FCS, 1% penicillin & streptomycin, 1% 200 mM glutamine. The plates was incubated at 37° C. for 24 hours in a humidified atmosphere containing 5% (v/v) CO_2.

[0157] The next day, compound dilutions were prepared according to a standard compound dilution protocol: Briefly, from a 5 mM stock, the compounds (estradiol, raloxifene, tibolone, and 4-hydroxy tamoxifen) were serially diluted in 100% DMSO from 5 mM to 0.00005 mM. Next, the compounds were diluted from 100% DMSO to into water by 1:25 to reduce the DMSO concentration to 4% by transferring 10 μl of compound solution into 240 μl of water. After mixing the compound solutions well, 10 μl of diluted compounds were added to each well of the 96-well plates. As such, the cells were exposed to a final compound concentration of 10 μM to 0.0001 μM. The 96-well plates were then incubated at 37° C. for 24 hours.

[0158] The lysis buffer containing probe linker solution and the denaturation oil were warmed to 50° C. The plate heat block/oven was set to 95° C.

[0159] The plates were removed from the incubator and spun down at 1200 rpm for 5 minutes to ensure that the cells will not lift off. The media was aspirated from the wells using the 8-channel aspirator, and immediately thereafter, 30 μl of lysis buffer/probe-linker solution per well were added, followed by 60 μl of denaturation oil per well. The plates were covered with foil plate cover and sealed. Then the plates were heated at 95° C. for 15 minutes.

[0160] Analysis of Gene Expression. The panel of signature genes used in this example included protein tyrosine phosphatase BAD2, Type II Hexokinase, multiple drug resistance gene, parvalbumin, prolactin, IL-18, N27, calpain, annexin I, and PKC subunit epsilon. Expression of the listed genes along with four housekeeping genes, i.e., actin, GAPDH, cyclophilin and L32, were analyzed using a cell lysate nuclease protection assay kit from High Throughput Genomics, Tucson, Ariz., following the manufacturer's protocol. Custom-made detection probes and linkers required to perform the assay had been obtained from the manufacturer.

[0161] Data Analysis. First, gene expression data were normalized using the housekeeping gene expression. Then, the expression level of each gene in the cell samples treated with estradiol, raloxifene, tibolone, and 4-hydroxy tamoxifen was divided by the corresponding gene expression level in DMSO treated control samples. The effect of the compounds estradiol, raloxifene, tibolone, and 4-hydroxy tamoxifen, respectively, on the expression of each signature gene is shown in **FIGS. 1A through 1E**. These expression profiles serve as reference expression profiles for the testing of candidate compounds.

7.2. Example 2

High Throughput Assay for Determining the Effect Candidate Compounds on Hot Flash Symptoms

[0162] The following example describes a high throughput assay for determining the effect of candidate compounds on the expression of a panel of genes whose expression level is associated with increase or decrease of hot flash symptoms. The rat pituitary cell line GH3 is used as the model system. Estradiol and Raloxifene at 100 nM are included in each experiment as reference compounds.

[0163] Treatment of GH3 Cells and Preparation of Cell Lysates. In 96-well V-bottom dishes, 50,000 GH3 cells per well are plated in 200 μl phenol red free Ham's F-12K media supplemented with 2.5% charcoal stripped FCS, 1% penicillin & streptomycin, 1% 200 mM glutamine. The plates are incubated at 37° C. for 24 hours in a humidified atmosphere containing 5% (v/v) CO_2.

[0164] The next day, candidate compound dilutions are prepared according to the compound dilution protocol described in Example 1, such that the cells are exposed to a final compound concentration of 10 μM to 0.0001 μM. The 96-well plates are then incubated at 37° C. for 24 hours.

[0165] As described in Example 1, the lysis buffer containing probe linker solution and the denaturation oil is warmed to 50° C. The plates are removed from the incubator and spun down at 1200 rpm for 5 minutes to ensure that the cells will not lift off. The media is aspirated from the wells using the 8-channel aspirator, and immediately thereafter, 30 μl of lysis buffer/probe-linker solution per well is added, followed by 60 μl of denaturation oil per well. The plates are covered with foil plate cover and sealed, and the plates are heated at 95° C. for 15 minutes.

[0166] Analysis of Gene Expression. Expression levels of the signature genes listed in Example 1, along with the expression levels of the four housekeeping genes used in Example 1, are analyzed using the cell lysate nuclease protection assay kit from High Throughput Genomics, Tucson, Ariz., following the manufacturer's protocol, and detection probes that had been custom-ordered from the manufacturer.

[0167] Data Analysis. After normalizing the gene expression data using the housekeeping gene expression, the expression level of each gene in the cell samples treated with candidate compounds is divided by the corresponding gene expression level in DMSO treated control samples. The effect of each candidate compound on the expression level of the panel of signature genes is compared with the reference profiles shown in **FIGS. 1A through 1E**.

7.3. Example 3

Assay for Determining Hot Flash Side Effects of an Osteoporosis Candidate Compound

[0168] The assays of the present invention may be used to screen compounds identified as potential therapeutic agents for the treatment of osteoporosis (or other medical conditions) for hot flash side effects prior to costly clinical trials. The compound is assayed as described in Example 1. The effect of the compound on the expression of the panel of genes listed in Example 1 is compared with the expression profiles of estradiol, tibolone, raloxifene and 4-hydroxy tamoxifen. A compound whose effect on the expression profile of the panel of signature genes mirrors more closely that of raloxifene and 4-hydroxy tamoxifen than that of estradiol and tibolone is likely to increase the incidence of undesirable hot flash side effects.

7.4. Example 4

Validation of Candidate Compounds Using a Rat Model of Hot Flash Symptoms

[0169] The following animal model is used to validate the effects of a candidate compound on hot flash symptoms.

[0170] Ovariectomized rats are treated for 8 or 9 days with candidate compounds. Rats are made morphine-dependent by implanting a morphine pellet (75 mg each) subcutaneously (sc) on days 3 and 5 of treatment. On the last day of treatment, a thermistor, connected to a data acquisition system, is placed on the tail of each animal and morphine addiction is withdrawn by naloxone injection (1.0 mg/kg, sc). Temperature measurements are taken for 1 h under ketamine (80 mg/kg, im) anesthesia. The candidate compounds that decrease the incidence of hot flash symptoms decrease the temperature of the rat tail in this model.

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aggcggatat ataccagggt ggggagttgc atttcagagt gagacggttc tgtcttcacc    540
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cagacttgtg acaggcactg gcggcccctc acatgatcct cagataccag attgtggcgt    780
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atgatcgcct cgcatatgat cgcctgctta ttcacggagc tcaaccaaaa ccaagtgcag   1680
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<210> SEQ ID NO 3
<211> LENGTH: 4254
<212> TYPE: DNA
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 3

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cttaacggaa gagcagacaa gaacttctca aagatgggca aaaagagtaa aaaggagaag    180
gagaagaaac ctgctgttgg catattcggg atgtttcgct atgcagattg gcttgacaag    240
ctgtgcatgg ctctgggaac tctcgctgct atcatccacg gaaccctgct tcccctcctg    300
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gctggggagc tcaacacccg tctcacagat gacgtctcca aaattaatga cggaattggt    660
gacaaacttg gaatgttctt tcagtccata acgacatttt cagccggttt tataatagga    720
tttataagtg gttggaagct aacccttgta attttggccg tcagccctct tattgggttg    780
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acacagacta gaggaaatga aattgaacca ggaaataatg cttatgaatc ccaaagtgac   2040
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ccatacagaa atgctttgaa gaaagcacac gtctttggga tcaccttcgc cttcacccag   2940
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gaactcatga cgtttgaaaa tgttatgttg gtattttctg ctgttgtctt tggtgccatg   3060
gcagcaggga ataccagttc attcgctcct gactacgcga aggccaaagt ctcggcatcc   3120
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cgacccaaca tcccagtgct tcagggactg agcttcgagg tgaagaaggg gcagacgctc   3300

cgcctggtgg gcagcagtgg ctgcgggaag agcacggtgg tccagctgct cgagcgcttc   3360

tacaacccca tggctggaac agtgtttcta gatggcaaag aaataaagca actcaatgtc   3420

cagtgcgtcc gcgcactggg cattgtgtcc caggagccca tcctgtttga ctgcagcatc   3480

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agagtgggag acaaagggac tcagctgtcg ggcgggcaga agcagcgcat cgccatcgcg   3660

cgcgccctcg tcagacagcc tcacatctta cttctggatg aagcgacatc agctctggat   3720

acggagagtg aaaaggtcgt ccaggaagcg ctggacaaag ccagggaagg ccgcacctgc   3780

gttgtgatcg cgcaccgcct gtccaccatc cagaacgcag acttgatcgt ggtgattcag   3840

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gttaaaggta atttcatcaa gacgagaagc cttcagagac ttcataatta aatgaaccga   4080

aattgaaaaa aaaatcatta aacagggcca catttttaa ttgtattatg tgattcaaga   4140

gaacatatag tttttttaaa aagaaatgtg tagttttgtt tcagtttttt taatttctac   4200

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<210> SEQ ID NO 4
<211> LENGTH: 1016
<212> TYPE: DNA
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 4

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agatgagaaa gcagtggttc tcttaggact tcttggggaa gtgtggtccc agtggtcatc    180

accatgaaca gccaagtgtc agcccggaaa gcagggacac tcctcctgct gatgatgtca    240

aaccttctgt tctgccaaaa tgtgcagacc ctgccagtct gttctggtgg cgactgccag    300

acacctctcc cggagctgtt tgaccgtgtg gtcatgcttt ctcactacat ccataccctg    360

tatacagata tgtttattga atttgataaa cagtatgtcc aagatcgtga gtttattgcc    420

aaggccatca atgactgccc cacttcttcc ctagctactc ctgaagacaa ggaacaagcc    480

cagaaagtcc ctccggaagt tcttttgaac ctgatcctca gtttggtgca ctcctggaat    540

gaccctctgt ttcaactaat aactggacta ggtggaatcc atgaagctcc tgatgctatc    600

atatcaagag ccaaagagat tgaggaacaa aacaagcggc ttcttgaagg gattgaaaag    660

ataattagcc aggcctatcc tgaagccaaa ggaaatgaga tctacttggt ttggtcacaa    720

ctcccatccc tgcaaggagt tgatgaagaa tccaaagact tggcttttta taacaacatt    780

cggtgcctgc gcagggattc ccacaaggtt gacaattatc tcaagttcct gaggtgccaa    840

attgtccata aaaacaactg ctaagcctac attcattcca tgtacatctg agatgttctt    900
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aaaagtctat ttcttcaaag gttctatttg cattacaact ttcagcacat gcttaagtat     960

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<210> SEQ ID NO 5
<211> LENGTH: 1908
<212> TYPE: DNA
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 5

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ctggccatgg tgatggaggt gggcatcctg gacgccgggg ggctgcgcgc gctgctgcga     180

gagcgcgccg ctcagtgcct gcttctggat tgtcgctcct tcttcgcctt caacgccggc     240

cacatcgtgg gctcagtgaa cgtgcgcttc agcaccatcg tgcggcgccg cgccaagggc     300

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gcctatacgc ccgtagtgct gctggacgaa cgcagcgccg ccctggacgg ccgcaagcgc     420

gacggcaccc tggccctggc cgcgggcgcg ctctgccgag aagcgcgctc cactcaagtc     480

ttcttcctcc aaggaggata tgaagcgttt tcggcttcct gccctgagct gtgcagcaaa     540

cagtccaccc ccatggggct cagcctcccg ctgagtacta gtgtgcctga cagtgcagaa     600

tccggatgca gctcctgtag caccccctctc tacgaccagg ggggcccagt ggagatcctg     660

tccttcctgt acctgggcag tgcttaccat gcttcccgga aagatatgct cgacgccttg     720

ggtatcactg ctttgatcaa cgtctcggcc aattgtccta accactttga gggtcactac     780

cagtacaaga gcatccctgt ggaggacaac cacaaggcag acattagctc ctggttcaac     840

gaggcgattg actttataga ctccatcaag gatgctggag gaagggtgtt tgtgcactgc     900

caggccggca tctccaggtc agccaccatc tgccttgctt acctcatgag gactaaccga     960

gtgaagctgg acgaggcctt tgagttcgtg aagcagaggc ggagtattat ctcccccaac    1020

ttcagcttca tgggccagct gctgcaattt gagtcccaag tactggcccc tcactgttct    1080

gcagaagctg ggagcccggc catggctgtc cttgaccggg gcacctctac tacaacggtc    1140

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gggaccaata tattgtgggt acatcgagcc cctctgacaa aacagggcag aagggaaagg    1500

actctgtttg agccagtttc ttcccttgcc tgtttttttct agaaacttcg tgcttgacat    1560

acctaccagt attaaccatt cccgatgaca tacacgtttg agagttttac cttatttatt    1620

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tacctcactt tttgtgtttg cgtactgtac tatcttgtaa atagacccag agcaggcttt    1740

cagcactgat ggacgaagcc agtgttggtt tgtttgtagc ttttagctat caacagttgt    1800

agtttgttta tttatgatct gaagtaatat atttcttctt ctgtgaagac attttgttac    1860

tgggatgact ttttttatac aacagaataa attatgacgt ttctattg                1908
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<210> SEQ ID NO 6
<211> LENGTH: 602
<212> TYPE: DNA
<213> ORGANISM: Rattus sp.

<400> SEQUENCE: 6

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aacccaggag ggccgcgaga agggctgaga tggggcatgg ggggtggaga ggtgggagac 180

ccaagcagtc agcgccactt agctttcggc caccagagtg gagaattctt caaccccaat 240

cttgccgtcc ccgtccttgt ctccagcagc catcagcgtc tttgtttcct tagcagacaa 300

gtctctggca tctgaggaga agcccttcag aatggacccc agctcatcct cctcaatgaa 360

gccacttttg tctttgtcca gaatgtggaa caccttcttc acatcatccg cactcttttt 420

cttcaggccc accatctgga agaacttttt gtggtcgaag gagtctgcag cagtaaaggc 480

tcctatcgcc ttcttgatgt cctcagcgct gagcaagtct gtcatcgaca tcctgcaact 540

tggatgacca gaagtcagag cctatataga aaagctgggc tggtgggaga tcccctcgtg 600

cc 602

<210> SEQ ID NO 7
<211> LENGTH: 1495
<212> TYPE: DNA
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 7

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ccaacattgg ccagaaggaa gactttgagg aagccaggaa gaaggcactg aagcttgggg 180

ccaaaaaggt gttcattgag gatgtaagca aggagtttgt ggaagagttc atctggcctg 240

ctgtccagtc cagtgcactc tatgaggacc gctatctcct aggcacctct ctcgccaggc 300

cttgcatagc tcgcaaacaa gtggaaattg cccagcgcga aggggccaag tatgtgtctc 360

acggcgccac ggggaagggc aatgaccagg tccgctttga gctcacctgc tactcgttag 420

caccccagat taaggtcatc gccccctgga ggatgcccga gttttacaac cggttcaagg 480

gccgaaatga tttgatggaa tacgcaaagc aacatggaat ccccatccct gtcaccccca 540

agagcccctg gagcatggat gagaacctta tgcacatcag ctacgaggct ggaatcctgg 600

aaaaccccaa gaaccaagca cctccaggtc tctacacaaa aactcaggac cctgccaaag 660

cacccaacac cccagatgtc cttgagatag aattcaaaaa aggggtccct gtgaaggtga 720

ccaacgtcaa agatggcact acccacagca catccttgga cctcttcatg tacctgaatg 780

aagttgcggg caagcatgga gtagggcgca ttgacatcgt ggagaaccgc ttcattggaa 840

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aattcgcaga gctcgtatac accggtttct ggcacagccc tgaatgtgaa tttgttcgcc 1020

actgcatcga caagtcccag gaacgggtgg aaggaaaggt gcaggtatct gtcttcaagg 1080

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gcatgaacgt acagggtgac tatgaaccca ttgatgccac cggcttcatc aatatcaact   1200

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caaagaggcc gggcctcccc gctctgcagc tctcccaggc tccagcatta attgttgtga   1320

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acctcccccc cacccacagg ctttgttccc tggtccccta tagcctacaa aagtggtcat   1440

cgaagggaag ggggggtggc aggcagctgc agaaagcgcg taaaatgaca attaa        1495
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<210> SEQ ID NO 8
<211> LENGTH: 2559
<212> TYPE: DNA
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 8

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gttcaggcgc agtatgggtc ctcgcctcaa ggagccagcc ccgcttctca gagctacagt    180

taccactctt cgggagaata cagctccgat ttcttaactc cagagtttgt caagtttagc    240

atggacctca ccaacactga aattactgcc accacttctc tccccagctt cagtaccttt    300

atggacaact acagcacagg ctacgacgtc aagccacctt gcttgtacca aatgcccctg    360

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gagcagagga agacacctgt ctcccgcctt tcactcttct cctttaagca gtccgccccg    660

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gctgtttgcg gtgacaacgc ggcctgtcag cattacggtg ttcgcacttg tgagggctgc    960

aaaggtttct ttaagcgcac ggtgcaaaaa aacgcgaaat atgtgtgttt agcaaataaa   1020

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tgcaatgggg tggtcctgca caggttgcaa tgcgtgcgtg gctttgggga atggattgat   1560

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ctttgcacac aggggctcca gcgcattttc tacctgaaat tggaagactt ggtaccacca   1860

ccagcaataa ttgacaaact tttcctggac accttacctt tctaagactt tctcccatgc   1920

acgtcaaaga actggaaaga aaaaaaaaat ccagaggggg ctggtcaaga tgggtagaga   1980

gctggctgaa gtgtccggtt catgtctccc ttctgtagac ccctagccct cacccctaaa   2040

gtaaacaaac aaacaagcaa acaaacaaac acaaattaaa aactgttgct atttcctaac   2100

ctgcaggcag aacgtgaaag ggcattttgg ctccggggca tcctggattt agaaaacgga   2160

caacatacac agtacagtgg tataaacttt tttattatca gttcaaaatc agtttattgt   2220

tcagaaggaa gattgcaaat gtatgatggg aaatgtttgg ccatgcttgc ttgttgcagt   2280

taagacaaat gtaaggcaaa tgtaacacac acacacacac acacacacac acacacacac   2340

acctcttaat gggaccctca tattttgccc tttaacaaga cttcaaagtt ttctgctgta   2400

aagaaagctg taatatatag taaaactaaa tgttgcgtgg gtggcatgaa ttgaaggcag   2460

aggcttgtaa attttatcca atgcagtttg gctttttaaa ttattttgtg cctatttatg   2520

aataaatatt acaaattcta aaaagtaagt gtgtttgca                          2559
```

<210> SEQ ID NO 9
<211> LENGTH: 628
<212> TYPE: DNA
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 9

```
atggctgcaa taccagaaga aggctcttgt gtcaacttca aagaaatgat gtttattgac     60

aacacacttt accttatacc tgaagataat ggagacttgg aatcagacca ctttggcaga    120

cttcactgta caaccgcagt aatgcggagc ataaatgacc aagttctctt cgttgacaaa    180

agaaacccgc ctgtgttcga ggacatgcct gatatcgacc gaacagccaa cgaatcccag    240

accagactga taatatatat gtacaaagat agtgaagtaa gaggactggc tgtgacccta    300

tctgtgaagg atggaaggat gtctaccctc tcctgtaaaa acaaaatcat ttcctttgag    360

gaaatgaatc cacctgaaaa tattgatgat ataaaaagtg atctcatatt ctttcaaaaa    420

cgtgtgccag gacacaacaa aatggaattt gaatcttccc tgtatgaagg acactttcta    480

gcttgccaaa aggaagatga tgctttcaaa ctcgttttga agaggaagga tgaaaatggg    540

gataaatctg taatgttcac tcttactaac ttacatcaaa gttaggtatt aaggtttctg    600

tattccagaa agatgattag cacacatg                                       628
```

<210> SEQ ID NO 10
<211> LENGTH: 1067
<212> TYPE: DNA
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 10

```
acggaggacc gtcagccagc aatcgcgtag ctcgatcgat cacccaggaa gagaaattgt     60

aagcagataa gaagaacgcg ttcggtttgg gacaccattt gcagctggaa atggggaatg    120

gactgtcaga ccagacttcc atcctatcca gcctgccgtc ctttcagtcc tttcacattg    180

ttattctggg tttggactgt gctggaaaga caacagtttt atacaggctg cagttcaacg    240
```

-continued

```
aatttgtaaa tactgtacct accaaaggat ttaacactga gaaaattaag gtaaccttgg    300
gcaattccaa aacagtcact tttcacttct gggatgtagg tggtcaagag aaattaagac    360
cactgtggaa gtcatatacc cgatgcacag atggcattgt gtttgtggtg gactctgttg    420
atgttgagag aatggaagaa gccaaaactg aacttcataa aataactagg atatcagaaa    480
atcagggagt ccctgtgctt attgttgcta acaaacaaga cctgaggaac tcactctctc    540
tctcagagat cgagaagttg ttagcaatgg gtgaactgag ctcatcgact ccttggcatt    600
tacagcccac ctgtgcaatc ataggagatg ggctaaagga aggacttgag aaactacatg    660
atatgataat taaaagaaga aaaatgttgc ggcaacagaa aaagaagaga tgaatggcag    720
tacttttata tcggtgtgga ataggtttta cttggtctga tttctgacaa gctgaagagt    780
gtctacagcc tggtttgcct gtctgccctc acggatgcta ttaaagcttt gttttgttga    840
acagtcagat acccaactct gttgccttgt ggaagatgag taaatgcaat gcttcttaaa    900
ggggtctctt ctccgtgacc cacaaatctt ttggtactac cattttggga agccaaaaaa    960
ggctagtaat tgaccagaaa acaattttgt ggaaatttga cctgaagtta gtgaaataaa   1020
actttgaaga gtgtaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaa                 1067
```

<210> SEQ ID NO 11
<211> LENGTH: 2641
<212> TYPE: DNA
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 11

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gccctggaag ccctgctttc ctgacaccct acaggactca caggcaggcc atggcagctc     60
tggcagcggg agtatccaag caacgtgcag ttgctgaggg gcttggctct aaccagaatg    120
ctgtgaagta tctgggccag gacttcgaga ccctgaggaa gcagtgcttg aactcggggg    180
tcctatttaa ggacccagaa tttccagcat gtccatcagc tttgggctac aaggatctag    240
gaccaggctc cccagacact caaggcatcg tatggaagcg acccacggaa ttgtgtccca    300
accctcagtt tattgttggt ggagccacac gcacagacat ccgccaaggg ggtcttgtgt    360
gggcacatgc gtctttgtga ttctctgctg gagaaacctg aacatgggct gaactgtagg    420
actcccttga tggtccaaga gactgctggc ttctagcagc cattgcctcc ctcaccttga    480
atgaaaagct gctttaccgg gtgcttccca gggaccagag cttccagaag gactacgcgg    540
gcatatttca tttccagttc tggcagtatg gagagtgggt ggaggtggtc attgatgacc    600
ggctccccac caagaacggg cagctgcttt ttctacactc cgaagaaggc aatgagtttt    660
ggagcgctct gctggagaaa gcctatgcca agctaaatgg ttcatatgag gctcttgttg    720
gaggctccac aatcgagggg tttgaggatt tcacaggtgg catctctgag ttttatgact    780
tgaagaagcc tccggaaaat ctgtactaca tcatccagaa ggccctccgc aaaggctctc    840
tgctgggctg ctccattgat gtctcaactg cagctgaagc agaagccacc accaggcaaa    900
agctggttaa gggtcatgca tactctgtta ctggagtcga agaggtgaat ttccatggcc    960
gtccagagaa gctgatcagg ttgaggaacc cgtggggtga agtggagtgg tcgggagcct   1020
ggagtgataa tgcacctgag tggaattaca tagatccaag gaggaaggag gagctggaca   1080
agaaagcaga agatggcgag ttctggatgt ccttttcgga tttcttgaag cagtactccc   1140
gactggagat ctgcaacctg tccccagact ctctgagcag tgaagagata cacaaatgga   1200
```

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```
acctagtact cttcaacggc cgctggacac ggggttctac agccgggggc tgcctgaact   1260
acccaggcac gtactggacc aacccccagt ttaaaatcca tttggatgag gtggatgagg   1320
accaggagga gggcaccagt gaaccctgct gtaccgtgct gctgggtctg atgcagaaga   1380
atcgaagacg tcaaaagagg atcggccagg gcatgctcag cataggctac gctgtctacc   1440
agattcccaa ggaggttctc agcacgatca ggagtgtaaa tagaccagca gggtccagat   1500
tcatagactt gggcccaggc tatgttccca gattgcagac tactggtctg tcctgccctt   1560
ggatcacaga tgcacgccac ttatcatgga ggggacggcc cagttggaga gtcacacgga   1620
tgcacacctt ggccgggact tcttcctggg acgccagccc tctacctgct ccagcactta   1680
catgaacctt cgggaggtgt ccagcagggt ccgactcccc ccgggacagt acctggtggt   1740
gccatccacc ttcgagccct tcaaggatgg tgacttctgc ttgagggtgt tctcagagaa   1800
gaaggccaag gctctggaaa ttggggatac tgtatctgga caccctcatg agccacatcc   1860
ccgtgacatg gatgaagaag atgaacatgt ccggagcctg tttgaggagt ttgtgggcaa   1920
ggattctgag atcagtgcta atcagctcaa gagggtcctg aatgaagtac tttctaaacg   1980
aacagacatg aaatttgatg gattcaacat caacacttgc agagaaatga tcagcctgct   2040
ggatagtgat gggacaggaa gcctgggacc tatggagttc aagactctct ggctgaagat   2100
ccgcacatat ctggagatct tccaagaaat ggaccataac catgtaggga ccattgaagc   2160
ccatgagatg aggacagctc tcaagaaagc aggtttcacc ctcaacaacc aggtgcagca   2220
gaccattgcc atgaggtatg cgtgcagtaa gcttggtgtt gacttcaacg gttttgtggc   2280
gtgtatgatc cgcctggaga ccctgttcaa actgttcagg cttttggata aggaccagaa   2340
tggcattgtc cagctctccc tggctgagtg gctgtgctgt gtgctggtct gacccgctgt   2400
ttggacatca acaacttccc tgtctcccac ttgtcccttt cagtcttatg aacatgtgac   2460
ctcaggtggc atttactgac tgttgtattg ttccagccaa ctgttgttcc tgagacactg   2520
cctttcccaa cagagcagtc tagggagccc cagatctctc agcagcaccg agctatgagc   2580
taactgggca gatcccaggg ttcagcagaa ggaaaagaat caattaaagt tgtgggccag   2640
g                                                                   2641
```

```
<210> SEQ ID NO 12
<211> LENGTH: 560
<212> TYPE: DNA
<213> ORGANISM: Rattus sp.

<400> SEQUENCE: 12

aaatgaaaca aggtttatgc cacacagtcc aacaatgtat aaagagcttg aagtagaaaa     60
gcttgtggaa aatgtatact ctttacatgg tacatacagt tttatagctc ataaataaac    120
acagagaaca ggagccattg ttcacattac cgaccagcaa cacagagcag cagtaacaac    180
acaaacacaa tttccttcga ctcagccaca aaaacttgaa atggtctcac catacttgag    240
gaaaatgaca ggtggtgaag tgttacagcc acacccacat caccaagtgc tttaagttgt    300
ctctgggtta gaaatatcaa caaccgtatt aactccagag agaagaaggc agggtaagac    360
cctgtcctat aaggcactta ataaattgac actacccttg tcttaaaata ctgcaaattt    420
gccttgaaat atgctgatgt tttacggcga aatttaggtt tcttttatcc aggtttagac    480
atagacataa tttgaataaa ttgataatac tgtctgatgg ccactcttta caccagcttc    540
```

-continued

```
aaaatggacc catttaaact                                              560


<210> SEQ ID NO 13
<211> LENGTH: 1081
<212> TYPE: DNA
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 13

gcttggaacg gtacgcgaag aaaagtgacc atggacaaca acgaaacctc cgtggattca     60

aaatccatta ataattttga aacaaagact atccatggaa gcaagtcgat ggactctgga    120

atatatctgg acagcagtta caaaatggat taccctgaaa tgggcttgtg tataataatt    180

aataataaga acttccataa aagcactgga atgtcagctc gcaatggtac cgatgtcgat    240

gcagctaacc tcagagagac attcatggcc ctgaaatacg aagtcaggaa taaaaatgac    300

cttactcgtg aagaaattat ggaattgatg gatagtgttt ctaaggaaga tcacagcaaa    360

aggagcagtt ttgtgtgtgt gattctaagt catggagatg aaggagtaat ttttggaacg    420

aacggacctg tggacctgaa aaaactaact agtttcttca gaggcgacta ctgccggagt    480

ctgactggaa agccgaaact cttcatcatt caggcctgcc gaggtacaga gctggactgc    540

ggtattgaga cagacagtgg aactgacgat gatatggcat gccagaagat accagtgggg    600

gccgacttcc tgtatgctta ctctaccgca cccggttact attcctggag aaattcaagg    660

gacgggtcat ggttcatcca gtcactttgc gccatgctga aactgtacgc gcacaagctg    720

gaattcatgc acatcctcac tcgtgttaac cggaaggtgg ccatggaatt tgagtccttc    780

tccctggacg ccactttcca cgcaaagaaa cagatcccgt gtattgtgtc aatgctcaca    840

aaagaactgt acttttatca ctaaaggaat gactgggagt ggggtagggg catgtttctg    900

ttttggtttt tttttggttt ttggtttgtt tttttttttt ttatttgaat gccaaatgag    960

aaaactgtca gggagacttt ttttttcccc tctcatttaa atcaaatccg atgttccagg   1020

tcgtcattga acaataccac tgcctgcaat gcagccacaa tacaatacct cagctttgat   1080

a                                                                  1081


<210> SEQ ID NO 14
<211> LENGTH: 387
<212> TYPE: DNA
<213> ORGANISM: Rattus sp.

<400> SEQUENCE: 14

gacatgtctc agaatttgct ttattaacag tatcttgctc tcacagctag atctttggga     60

ttggatagga ttcaatcagt aggtggtgtc ggagctcctt cttctggctt cattgtgata    120

actttttgaa gaaaacttcg aattcttcat agctaacttc tccatcaccg ttcttatcca    180

gctctttaaa gagattgtct agagtacttg aagccttcag gaggctgggg aactctgact    240

gaatcagcag cttcagctcc tccttggaca gctggtttgg atcgccttct ttggctgcat    300

atttttgaaa aatgctcttc atttcttcgg gagatttctt agcgctcatt tttctgtgct    360

gcttgctgtc agtgagtgct gccagag                                        387


<210> SEQ ID NO 15
<211> LENGTH: 2668
<212> TYPE: DNA
<213> ORGANISM: Rattus norvegicus
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<400> SEQUENCE: 15

```
gagtggccag gagcaaagtc tggaagccgg cgagccacgg agcagagcac acagagctat     60
gaggcttgca caaatgtgtc tggccttcgg ctgggcagct gtcatattgg ttctacagac    120
ggtagacacg gcatctgctg tgaggactcc ttatgacaaa gcgagggtat ttgcagacct    180
gagcccccag gagataaagg ctgttcacag cttcctgatg aacagggagg agctggggct    240
gcagccgtcc aaggaaccga ctttggccaa aaactctgtg tttctcattg agatgctact    300
gcccaagaag aagcatgtgt tgaaatttct ggatgaagga agaaaaggtc ctaaccggga    360
agctagggct gtcatcttct tcggtgccca ggactacccc aatgtcactg agtttgctgt    420
ggggcccctg ccacggccct actatattcg agcactatcc cccaggccag ggcaccatct    480
gtcctggtca tccaggccca tctccacagc agagtacgac ctcctctacc acacgctgaa    540
gagagccacc atgcctctgc accagttttt ccttgacacc actggcttct cattcctagg    600
ctgtgacgac cgatgcttga cttttactga cgtagctcca cgtggtgtgg cgtctggtca    660
gcgtagaagc tggtttattg tgcagcgcta tgtggaaggc tatttcctgc atcctacagg    720
gctggagatc ctattggatc atgggagcac agatgtccag gactggagag tggagcagct    780
ctggtataac ggcaagttct acaacaaccc agaggaactg gctcggaaat acgcagttgg    840
agaagtggac acggtggtcc tcgaggaccc actgcccaat ggcacagaga agcccccact    900
cttttcttcc tacaaacccc ggggggaatt ccatacacca gtcaatgttg ctggccccca    960
cgttgtccag cccagcggcc cccgatataa actagagggc aacactgtgc tctatggagg   1020
ttggagcttc tcttatcggc taagatcctc ttctgggctg cagatcttca atgtgctctt   1080
tggaggtgag cgtgttgcct acgaggtcag tgtgcaggag gctgtggcac tgtatggagg   1140
acacacacct gcaggcatgc agactaagta cattgatgtt ggctggggcc tgggcagtgt   1200
cactcacgag ttggcccctg gcattgactg tccagagact gctactttcc tggatgcctt   1260
ccactattac gacagcgatg gccctgtcca ttatccacat gctctgtgcc tctttgagat   1320
gcccacaggg gtgcccctga ggcgccactt taactcaaac tttaaaggtg gcttcaactt   1380
ctatgcgggt ttgaagggat atgtgctggt gctacggacg acatcaactg tctataatta   1440
tgattacatc tgggatttca tcttctactc taacggggtg atggaggcca agatgcacgc   1500
cactggctat gtccatgcga ccttctacac tcctgaggga ctgcgccatg gcactcgctt   1560
acaaacccac ctgcttggta acatccacac ccacctggtg cactaccgtg ttgacatgga   1620
cgtggcaggc accaagaaca gcttccagac actgacgatg aagctggaaa acctcaccaa   1680
tccctggagc ccaagtcact ccctggtcca gcccacactc gagcagaccc agtactccca   1740
ggagcaccag gctgcattcc gcttcggaca gactctgccc aagtacctgc tctttagcag   1800
cccccaaaag aactgctggg gtcacaggcg cagctaccgc ctgcagatcc attctatggc   1860
tgagcaggtg ctgccaccag gctggcagga ggagcgggct gtcacttggg ccaggtatcc   1920
tttggctgta acaaagtatc gggaatctga gcgctacagc agcagcctct acaaccagaa   1980
tgacccctgg gatcccccg tggtctttga ggagttcctt cggaacaacg agaacattga   2040
agatgaggat ttggtggcct gggtgacagt gggcttcctg cacatccctc actcagaaga   2100
tgtccccaac acagccacac ctggaaactc tgtgggcttc ttgctccggc ccttcaactt   2160
cttcccagag gacccatccc tggcttctag agacactgtg atagtgtggc cccaggacaa   2220
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gggcctcaac cgtgtccagc gctggatccc tgaggacagg cgctgcttgg tgtctcctcc   2280

tttcagctat aatgggacct acaagcctgt gtgatgggtc agccccagcc tctgcagcac   2340

acccagagcc tcacaaagac agggaaaaca aacaaacgaa acttctgtct ctaccctgta   2400

tgctaccttt tagctctact cgtgtttcat taccatacct gccacagact tccaagacca   2460

ttgcaaagga aggaccacaa ttttctgccg gtggttcggt ggtttggagg gtggtgtttt   2520

ttaaatattt gaggtgttta tcttcctatc aggagacttt agagaaattg tctcacctca   2580

gtgatgggag ggagtaagga aactgtcata tgaaaatcta ataaaatgac acctttgctc   2640

tttagacatc aaaaaaaaaa aaaaaaaa                                      2668
```

<210> SEQ ID NO 16
<211> LENGTH: 1140
<212> TYPE: DNA
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 16

```
ggatcctaac ttcttcccaa gcaccagtca ctggagacat aggagaggca agaagcctct     60

gggcaactga taaggattcc ccgcacccag gggaggcgat ccccaagccc agcctctcag    120

ggtcctgaca agccatgctg cggagagcaa tcacaggcca gccaatgaca gctgctccaa    180

gggcgtgtcc ttacctcctg cacatataaa tgaaacttgc tgcggggcca atacattctc    240

attctgatag actcaggaag caatcatggt gctctctgca gctgacaaaa ccaacatcaa    300

gaactgctgg gggaagattg gtggccatgg tggtgaatat ggcgaggagg ccctacagag    360

gtaacatcag gaccctgttc tttaaggaca gcaggatcca aaccggacca gggactcagt    420

gggtagctcc taagtgtgct ttcccgtggc ctcaacttat ctctccttct cacaggatgt    480

tcgctgcctt ccccaccacc aagacctact tctctcacat tgatgtaagc cccggctctg    540

cccaggtcaa ggctcacggc aagaaggttg ctgatgccct ggccaaagct gcagaccacg    600

tcgaagacct gcctggtgcc ctgtccactc tgagcgacct gcatgcccac aaactgcgtg    660

tggatcctgt caacttcaag gtgtgagctc agacctggca gggggcacct gggaccttca    720

aggatccctt ggggcagtgg tgaggggcac agggggaggg ggaaggggtt cctcatgccc    780

agggcaggga acacagtgtt ccaggaaggg gagcttaccc agcagggtgt ctactactag    840

ggactgaccc ttcctgtctc tgcagttcct gagccactgc ctgctggtga ccttggcttg    900

ccaccaccct ggggatttca cacctgccat gcacgcctct ctggacaaat tccttgcctc    960

tgtgagcacc gtgctgacct ccaagtaccg ttaagccacc tcctgtcggg cttgccttct   1020

gaccaggccc ttcttccgtc ccttgcacct gtctttgaat aaagcaaaag taggaagaat   1080

cccgtgtgcc tgtttctcac atgtgcaaag gtgacaatgt ttggtatggg aaaatcctcc   1140
```

<210> SEQ ID NO 17
<211> LENGTH: 626
<212> TYPE: DNA
<213> ORGANISM: Rattus sp.

<400> SEQUENCE: 17

```
gtcttataaa attgttttat tttattctat gtaaacttct ttctcaggaa tggtaccaaa     60

gtgacttgct tacagggatt tataatttgg gataaacata attaacaaaa caagtatttc    120

tctattgtac agatgaatga tctaaaacat atttataggc tacattattc aaagaaggta    180
```

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attagactta ctgacaaagc aactttccta cttaagcctt gcaataggaa gaaaacaacg    240

gctaagagat gttctcctcg gaatcttaca gagcagttgg gatgtttagt ttcctccaca    300

cagagccacc aggatttttt catagtctcc tttggtttca tccaggatgg cttggcagag    360

agggattccg tacttcttct ggtaaaatac tttgatttca ttcatgtcaa tttccgaacg    420

ggagaccata atcctgatca atgtcttatg gcgagttcca gcacccttca tggcttcata    480

cagtttctca gcaaagaaag ctggagtgct ggtggcacac ttcacaatgg ttgtgaggca    540

cttctcaatg tcacccttca gttccagatc caaggctttg ttcatgtcat gttgactgta    600

ctttctataa ttctgaaaca ctttcc                                        626

<210> SEQ ID NO 18
<211> LENGTH: 508
<212> TYPE: DNA
<213> ORGANISM: Rattus sp.

<400> SEQUENCE: 18

gccattaaaa aactttattt tgattttttt ttttttacaa aggatagccc cactttgggg     60

tgaaaatata tttggttaag tacaaaatat agtttttaat catacaaaaa gatacacaaa    120

atacaaaaac aacaaagacg acacctccat cagctcacac tttctggctg cacagttctc    180

gggtggagtg cttagagtgg agcttggagc tctgacacag gacacggaac aaagtgctaa    240

ggcggagtaa gttttggagt gctagaggcc acggcgagag cagaactttc tcctaacact    300

ggacaggaaa tgggtctggg agcttccatg gaaacctgca gttctcactg tgcctccatc    360

actgttaggg catctcaatg taagagtgtc accagggtcc agctgtcagc agcaaccctt    420

catatgagta gtgtcagaat cacagaagtc tgtgtgttgg gggaaagcct ccaaaagtaa    480

taaccaagct gggcctttaa cctcagca                                      508

<210> SEQ ID NO 19
<211> LENGTH: 2642
<212> TYPE: DNA
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 19

agtgtgctct aaaggcgacg ggtttgtcgg agcaccataa tatggtgtgg gaagtgaaga     60

cgaaccagag gcctcatgca gtacagagac tcctgctggt gatggacgag agagctacag    120

gagtgagtga ctcattggag ttgctgcagt gtaatgagaa tgtgccgtcc tctccaggat    180

acaactcctg tgatgagcac atggagcttg atgaccttcc tgaacttcag gctgttcaaa    240

gtgaccctac ccaatctgcc atataccagc tcagttcaga tgtatctcat caagaatatc    300

caagaccatc ttggagccag aatacctcag acataccgga aaatactcac cgtgaagatg    360

aggtggactg gctaacagag ttagcaaata ttgccactag tccacaaagt ccactaatgc    420

agtgctcatt ttataacaga tcatctcctg tacacatcat agctactagc aacagtttac    480

attcctatgc acgcccacca ccagtgtcct ctgcaaagag cggaccagcc ttccctcatg    540

accactggaa ggaagaaaca ccagtaagac atgaaagggc aaacagtgag tcagagtcag    600

gaattttctg catgtcctcc ctgtcagatg atgatgatct gggctggtgc aattcctggc    660

catcaaccgt ttggcattgc tttctcaaag gcacacgact gtgcttccat aaggaaagca    720

aaaaggagtg gcaagatgtt gaagactttg ctagagccgc cacctgtgat gaggaggaga    780
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tccaaatggg cactcacaag ggctatgggt ctgatggtct aaagttgtta tcccatgaag    840
agagtgtgtc attcggtgag tctgtactga agttgacatt tgatcctggt acagtagaag    900
atggcttgct tactgtggag tgcaagctgg accacccttt ctatgttaaa aataaaggct    960
ggtcatcatt ttatccaagc ttgactgtgg tacagcacgg cattccatgc tgtgagattc   1020
atattggtga cgtatgtcta cctcctggac accctgatgc cattaatttc gatgattcag   1080
gtgtttttga tacatttaaa agctatgact tcacacctat ggactcttcc gcagtctacg   1140
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gtcaagagtt tgcaggatct gaattcagta aaagctgtgg ttcaccagga tcatcacagc   1260
tctcctctag ttctttatac actaaagctg tcaaaagcca cagctcaggg actgtgagtg   1320
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acagagttga atatactcag atgtatccag ggaaagataa cagagccata agtgtgatcc   1440
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gtctcaccat ttgtcctaca tttgtgtgac cataaaatgc tgggagcatt gagttttgta   1740
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atcaacattg taaaccaaat tgccttattt tccttccaaa cttcatatat gtctatcagg   1860
taatataggc ttgaaaattg atatcctgtg gtgctaaagt acagtagaaa gagagatgtg   1920
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tacatcagct ccttactgct tattaatctg tattgtaccc acgatatcag gaatgaagta   2040
gaagctggcc tttccatgaa tagccaccac atggcccagt gtctctgcca aacacagttg   2100
cttctagtct ggccagtgcc aagagacaac ataacatggc aggtggatgg tgtcattgtc   2160
tcagctgaga gccacctgct tcagatacca tgggcgtgtg ctgagtggat gcaccagggg   2220
gcagcacagc tccatgattc ccagtggggg ccaagccagg ctaaggtgca gtcagttagc   2280
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tgcagtgcaa cacttgcact ttacccctcc ggcttctaca gtagagcaag tccttcggca   2460
gccctgctgc tttcgctctg cgctacaatc atggcttctc atgttactta ccaagtggta   2520
tttttggtta ggaatcacag ctgtaaagtt gatttcagtt cattacactt cttaaacata   2580
ttgcccctca attttgcaca ctatattctt gtatattatt tcaaataaaa tgaaaaaagt   2640
ct                                                                  2642
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<210> SEQ ID NO 20
<211> LENGTH: 1671
<212> TYPE: DNA
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 20

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aggagcgcgc gcctgtgtcc cttggggacc cacagttgca aagagacagc tgcttgattt    120
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ggtcacccac tcgcccagac tataggagcc tcccgggaca ctcttgagtt gcacctttct    180

gcagagcaga ctgtttgagc cccgcagtg ctcgtaggat ttgaagcgtt ctgatactgg    240

aggcttgctc gccgcgctcg cccgactctg ggacacattt gtccgagcca caggagtgtg    300

cgggtgactg acatatcatc tcttcgactc tccatgagct catgaacctg ggagcaggtg    360

cccggaaatg gcgcggcctc tgagcgacag gaccccgggc cccctgctgc tgggtggccc    420

ggctggggcc ccccctggcg ggggagcgct gcttgggctg aggagccttc tgcagggaaa    480

cagcaagccc aaagaaccgg ccagctgtct cctgaaggaa aaggagcgca aggcaactct    540

gccctcagcc cccgtcccgg gacccgtcct ggaaacggcg ggcccagcgg atgccccaac    600

tggggcggtt agtggcggtg ggtcccctcg ggggcgctca gggcctgtgg ctggcccgag    660

tctttttgcg ccgctgctgt gggaacgcac tttgcctttc ggggacgtgg aatacgtgga    720

cctggacgcc ttcttgctgg agcacgggct accgccgagc ccgccgcccc ccgggggcct    780

gtcgccggca ccctctccag cgcgcactcc cgcgccctcc cccgggcccg gctcttgcag    840

ctcctcttcc ccccgctcct cgcccgggca cgcccccgcg cgggccactc tgggagccgc    900

cggcggccac cgcgcaggct tgacctctag ggacacaccc agtcctgtgg acccagacac    960

cgtggaggtg ctaatgacct ttgaacctga tccggctgat cttgccctgt caagcattcc   1020

aggccatgag acttttgacc ctcggaggca ccgcttctca gaggaggaat tgaagcctca   1080

gccaatcatg aagaaggcaa ggaaagtcca ggtgcccgag gaacagaagg atgagaagta   1140

ctggagccgg aggtacaaga acaatgaagc agccaagagg tcgagagacg caagaagact   1200

caaggagaac cagatatctg tgcgggcagc cttcctggag aaggaaaacg ccctattgcg   1260

gcaggaggtg gtggctgtgc ggcaggagct gtcccactac cgtgctgtgc tttcacgcta   1320

ccaggcccaa cacgggacac tgtgaggcca cttccaccct gccagggcag agtcctgttc   1380

cttgttcaga cttacacctg acaccccct tcctccttgt cccatggcca atggtctggc   1440

cagctaggtg cccaggaacg tcatgatgca gacaaataca tttatatttt taagaaaaag   1500

ctagccttcc cccacctccc ttgtgggggt ggggagggtc ctgtgtgcac ttttagcatg   1560

tcgaggaacc catccatcca accgcctcca tcaacacaat cctgaataaa tcttgagaac   1620

ccaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaagaatt c            1671
```

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<210> SEQ ID NO 21
<211> LENGTH: 2249
<212> TYPE: DNA
<213> ORGANISM: Rattus rattus

<400> SEQUENCE: 21

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ccgagggctt catggatccg ccaagatcat caacacccag ggactgggac cagatgaagg    120

agggagaagg cagcgaggag aagttgagga ggaggatgaa gatgaggagg aagaggatga    180

ggaggaggag gaagaggatg aggaggagga ggacttgtct tctcctcaag ggctacctga    240

gcctctggag aatgtggaag tcccctctgg accccaggtc ctcacagatg gcccccggga    300

acatagcaag agtgctagcc tcctatttgg catgcgaaac agtgcagcca gtgacgagga    360

ctcaagctgg gccaccttat cgcagggcag cccctcctat ggctctccgg aggacacaga    420

ttccttctgg aaccccaacg ctttcgagac ggattccgat ctaccggctg gatggatgag    480
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ggtccaagac acctcaggga cctactactg gcacatccca acagggacca cccagtggga    540
acccccaggc cgggcctccc catcacaggg gaacagtccc caagaagagt cccagctcac    600
ctggactggc tttgctcacc aagaaggctt tgaggaagga gagttttgga aggatgaacc    660
tagtgaagag gccccaatgg agttgggact gaaggaccct gaggaggggga cattgccctt    720
ctcagctcag agcctcagcc cagagccagt gccccaggag gaggagaatc tgccccaacg    780
gaacgccaat ccagggatca agtgtttcgc tgtgcgctcc ctaggctggg tagagatgac    840
tgaggaggag ctggccccag gacgcagcag tgtggcagtc aacaattgta tccgccagct    900
ctcctaccac aaaaacaatc tacatgatcc gatgtctgga ggctggggag agggaaagga    960
tctgctgctc cagctggagg atgagacgct aaagttggtg gagccacaga accagacact   1020
gctgcatgcc cagcccatcg tcagcattcg cgtgtggggc gttgggcggg acagtggaag   1080
agagagggac tttgcctacg tagctcgaga taagctgacc cagatgctca agtgccacgt   1140
gtttcgctgt gaggcacctg ccaagaacat cgccaccagc ctgcatgaga tctgctccaa   1200
gatcatgtct gaacggcgca atgctcgctg cttggtaaat ggactctccc tggaccactc   1260
taaacttgtg gatgtccctt tccaagtgga attcccagca ccaaagaatg aactggtgca   1320
gaagtttcaa gtctattacc tagggaacgt gcctgtggct aaacccgttg ggtagatgt    1380
gattaatgga gccctggagt cagtcctgtc ttccagtagt cgtgagcagt ggaccccaag   1440
tcacgtcagc gtggcccctg ccaccctcac catcttgcac cagcagacag aagcagtgct   1500
gggggagtgt cgagtgcggt tcctctcctt cctggctgtg ggcagagatg tgcacacgtt   1560
cgcattcatc atggctgccg gcccagcctc cttctgctgt cacatgtttt ggtgcgagcc   1620
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tctggatgct cgttcccaga cctccacctc ctgcctccca gcacccccctg cggagtcagt   1740
tgcgagacgt gtagggtgga ctgtccgcag gggtgttcag tctctgtggg gttccctcaa   1800
gcccaaacgt ctaggatccc agaccccatg aagagctccc atcctccctc tacctgcttg   1860
ttttgggccc cagggaactc aagggtttgg ggcaggtagg ggctgtggat gttcttccta   1920
caccccatgt cccccgggct gcccctcctc agtagctggg gttccctacc taggggctgg   1980
gagagagaaa ttgaattcct tcacagaagt cattacactg gactgatgac atcggggccg   2040
agaagcaaag ccagccttgg gctctccatc cccagtgttt gaggtggagc aggaggaact   2100
ggtccaagcc aggccccatc ctcaaaggcc caataagggc aggaggggac tggtctcggc   2160
atggatcccc tgtcccctgt tctgcggaca cctccactgt actgatatca ctaataaagt   2220
ctgtctgtcg ctgaaaaaaa aaaaaaaaa                                     2249
```

<210> SEQ ID NO 22
<211> LENGTH: 3113
<212> TYPE: DNA
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 22

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cctagatgcc ccagtcttct actgctctga ccccacccgc tttctcccgg ctcggtacag     60
ctggtgccgg ggggtgctgc tttctgccct gcgctgcgca ccgttagtgc cctgcccctg    120
tccttccgat ctcagagtct gcggagtgcc cctatcgccg tccacctgtt tcctcagaaa    180
aaaggccagc tcgtgatccc tgctgcgttc ctggggccat ggcgggtctg ggtcctggcg    240
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ggggcgactc agaaggggga ccccgacccc tgttttgcag aaagggggcg ctgaggcaga    300
aggtggtcca cgaggtgaag agccacaagt tcaccgctcg tttcttcaag cagccaacct    360
tctgcagtca ctgtaccgac ttcatctggg gcattggaaa gcagggcctg caatgtcaag    420
tctgcagctt tgtggttcac cgccgatgcc acgaatttgt gaccttcgag tgtccaggag    480
ctggaaaggg cccccagacg gacgaccctc gcaacaagca caagttccgt ctgcacagct    540
acagcagtcc caccttctgc gaccactgtg gttccctcct ctacgggctg gtgcaccagg    600
gcatgaaatg ttcctgttgc gaaatgaatg tgcaccgacg ctgtgtgcgc agcgtgccct    660
ccctttgcgg cgtggaccat acagagcgcc gtggacgtct gcaactggaa atccgggctc    720
ccacatcaga tgagatccat attactgtgg gtgaggcccg gaacctcatt cctatggacc    780
ccaatggcct gtctgatccc tatgtgaaac tgaagctcat cccggaccct cggaacctga    840
caaaacagaa gacaaagacc gtgaaagcca cactgaatcc cgtgtggaac gagaccttcg    900
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aggctcctgt ggatggatgg tacaagttac tgaaccagga ggagggcgag tattacaatg   1080
taccggtggc cgatgctgac aactgcagcc tcctccagaa gtttgaggcc tgtaattacc   1140
ccttggaatt gtatgagaga gtgcggatgg gcccctcttc ctctcccatt ccttctccat   1200
cccccagtcc cacggactcc aagagatgct tcttcggtgc cagcccagga cgcctgcata   1260
tctctgactt cagcttcctc atggttctag ggaaaggcag ttttgggaag gtgatgctgg   1320
cagagcgcag aggatccgat gaactctatg ccatcaagat actgaaaaaa gacgtcattg   1380
tccaggatga tgatgtagac tgcaccottg tggagaagcg tgtgctggca ttgggaggcc   1440
gaggtcctgg aggccggcca cactttctca cacaacttca ttccaccttt cagactccgg   1500
accgcctgta ttttgtgatg gagtacgtca ctgggggcga tttaatgtac cacattcagc   1560
aactgggcaa gtttaaggag ccccacgcag cattctatgc cgcggaaatc gccataggcc   1620
tcttcttcct tcacaaccag ggcatcatct acagggacct caagttggat aatgtgatgc   1680
tggatgctga aggacacatc aagatcacag acttcggcat gtgtaaagag aatgtcttcc   1740
ctgggtccac aacccgcacc ttctgtggga ccccagacta catagcacct gagatcattg   1800
cctatcagcc ctatgggaag tctgtcgact ggtggtcctt tggagtcctg ctgtatgaga   1860
tgttggcagg acagccaccc tttgatgggg aagatgagga ggagctgttt caagccatca   1920
tggaacaaac tgtcacctat cccaagtcac tttcccggga agctgtggcc atctgcaagg   1980
ggttcctgac caagcaccca ggaaagcgcc tgggctcagg gccagatggg gaacccacca   2040
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cgcctccttt tagaccacgt ccgtgtggcc gcagcggcga aaactttgac aagttcttca   2160
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ctgatttcca gggctttact tatgtgaacc cggacttcgt gcacccagat gcccgcagcc   2280
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gtgctccctc cgccaagttg gctgtaactc ccatccaccc ccatccccgc ctctagtccg   2400
aattttaggt ctcttaaacc acccaacctt ctggcctctt tcacgcgccc caagtgggtt   2460
ctagacgctg ttccccagca ttgctggcat tttaaacttc aaacagtctc tagggccttt   2520
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ctgtgttcta ggttcgttgt gctgagccct ggtttttccc cacccccaac atctggatgc 2580

tgttccaact cttcccagaa accccactcc gtgtggggtt ctagactcta tcttggtagt 2640

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cggaatcaca cacacacaca cacacacaca cacacacaca cacacacaca cacacacacc 3000

ccttgtcctc cgcagtgcct gccactttct gggactttct catcccccac gcccttcctt 3060

tatcctctcc cacccagaca cagctgctgg agaataaatt tggagctctc gag 3113

<210> SEQ ID NO 23
<211> LENGTH: 3611
<212> TYPE: DNA
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 23

cccgcggggg aagacgcacg ggcgggctcg gctctcccgg ggagcggccc gggactgcac 60

cgggaccggc gcctccccgc tccggctgcc ctcggcctcg ccccgggccc gggcggatga 120

gccgggggcc cgggggggaca tggaagcgct gacgctgtgg cttcttccct ggatatgcca 180

gtgcgttacg gtgcgggccg actccatcat ccacatcggt gccatcttcg aggagaacgc 240

agccaaggac gacagggtgt tccagttggc tgtatcagac ttgagcctca atgatgacat 300

cctgcagagt gagaagatca cctactccat caaggtcatc gaggccaata atccgttcca 360

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gggctgtgca tctgccaacg ccctgcagtc cctcacagac gccatgcaca tcccacacct 480

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acaaaaggtg gacaagaaca tcagtcacgt gttcaccagc ctcttcacca ccatgaagac 780

ggaggagctg aatcgctacc gggacacact gcgccgggcc atcctcctgc ttagcccaca 840

gggggcgcac tcgttcatca acgaggctgt ggagaccaac ttggcttcca aggacagcca 900

ctgggtcttc gtgaatgagg aaatcagtga ccccgagatc ctggatctgg tccacagtgc 960

ccttggcagg atgaccgtgg tccggcaaat cttcccatct gcaaaggaca accagaaatg 1020

catgaggaat aaccaccgca tctcttccct gctctgtgat ccacaggaag gctacctcca 1080

aatgctgcag atctccaatc tctatctgta cgacagtgtt ctgatgctgg ccaacgcctt 1140

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ggctaaagct ctcggattca aatacgagat ataccaggcc cctgatggca ggtatggcca   1620
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cttggccatc tctgctatta ccatcacccc ggagagagag agcgttgtgg acttcagcaa   1740
gcgatacatg gactactcag tggggatcct catcaagaag ccggaggaga aaatcagcat   1800
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gaatggaggg gctgacaact gtgtgtccaa tccttcagaa ggtatcagga aggcaaagaa   2340
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cgactgctca gtgactgtca tcggcaacag catcagcagc aagggctatg ggattgccct   2460
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aggggacctg gatgtgctca agcagaagtg gtggccacac acaggccgct gcgacctcac   2580
cagccattcc agtgcacaga ctgatggtaa atcccttaag ctgcacagct tcgctggggt   2640
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gctgagcagc tcagccacca tgccctcaat tcagtgcaaa cacaggtcgc ccaatggggg   3060
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caacacagac cacacacaca cacacacaca cacacacaca cacacacaga ctctttcatt   3300
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attcccaatt ttcgaagtca gtaaaaaaaa acaaaacaaa acaaaaactg tatgaatgac   3420
tttgtaaatt ttgttctata tgaataaaaa ggcaaattac ttgtgatcta ttttatttct   3480
gtgagtgggg taggcaggcc agtacagagg ggattgatct gcaggtgctg gtcctgcagg   3540
gtccaagtgg ctcccccttc ctggttgcat gaccctgtgc caggatgccg ggacatcggt   3600
ttgcaggtcc g                                                        3611
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<210> SEQ ID NO 24
<211> LENGTH: 4422
<212> TYPE: DNA
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 24

gatcctggga gagtccgacg ttcgccggct aacgcaggac aggtcactga ggtccgccag 60

gtgtgtccgc cgtttgtttc ctgcccctcg ccaggttcgg tgtccgctgc cgaggcccga 120

ggcccttctt ccgctgtcgg cgcgcccccc tgtctcgttt cggccccgcc gggacaacgc 180

ctggagtcgc agcagccggg tgatcgcgcc cgtcatcctc taccaacgtt tccaggctgc 240

cctgctcggg agtgggtgag aatgaagcta aaggagatag accgcacagc gatgcaggcg 300

tggagccctg cccagaacca ccccatttac ctggccacag ggacatctgc tcagcagttg 360

gacgcgacat tcagtaccaa cgcttccctt gagatattcg agctcgacct ttccgacccg 420

tccttggata tgaagtcctg tgctactttc tcctcgtctc acaggtacca caagttgatt 480

tggggacctc ataaaatgga ttccaaagga gatgtttctg gagttttgat tgcaggtgga 540

gaaaatggaa acattattct ctacgatcct tctaaaatta tagctggaga caaggaagtt 600

gtgattgccc aaaaggacaa gcatactggg ccagtgagag ccttggatgt aaacatcttc 660

cagaccaatt tggtggcctc tggtgctaat gaatctgaaa tctacatttg ggatctaaac 720

aattttgcaa ctccaatgac gccaggagct aaaacacagc caccagaaga tatcagctgc 780

atagcctgga acagacaggt ccagcatatc ttagcatcag ccagtcccag tggccgggca 840

acggtctggg atctcaggaa aaacgagcct attatcaaag tcagcaacca cagtaactgg 900

atgcactgct ctgggctggc atggcaccct gatgtcgcta ctcagatggt ccttgcttct 960

gaggatgatc gattaccagt ggtccagatg tgggatcttc gatttgcttc ctctcccctc 1020

cgggttctgg aaaatcatgc caggggggatt ttggcaattg cttggagcat ggcagatcct 1080

gaattgttgt taagctgtgg aaaagatgct aagattctct gctctaaccc taacacggga 1140

gaggtattat atgaacttcc caccaacaca cagtggtgct ttgatatcca gtggtgtcct 1200

cggaatcctg ccgtcctgtc tgccgcttcc ttcgatggtc gcatccgtgt ctactccatc 1260

atgggaggga gcatagacgg cttaaggcag aaacaagttg acaagctttc gtcgtctttt 1320

gggaatcttg atccgtttgg cacaggacag ccgctccctc cattacaaat cccacagcag 1380

acttcacagc acagcatcgt gctgcccctg aagaagccgc ccaagtggat ccggagacct 1440

gtgggcgcct ccttctcatt cggagggaag ttggtgactt tcgagaacgt tacagggcag 1500

cctcagcagg gagctgagca gccacggcgg cagcccgtgt tcatcagtca ggtggtgaca 1560

gaaaaggact tcctcagccg ctcagagcag ctacagcacg tcgtcgagtc tcagggcttt 1620

atcagttact gccagaaaaa gatcgatgcg tcccagactg acttcgagaa gaatgtgtgg 1680

tccttcttga aggtaaagtt tgaggaggat tctcgtggga aataccttga gcttcttgga 1740

tatagaagag aagatctggg agagaagatt gctttggcct tgaacagagt ggacggatcc 1800

gatgtggctc ttaaggactc tgaccgcgta gcgcagagtg atggggagga gagccctgcc 1860

gaggaagggc agctcctggg agagcgcatt aaagaggaaa aacaggagtg tgactttctg 1920

ccctcggctg gcggtgggac ctttaacatc tccgtcagtg gggatattga tggtttaatt 1980

acccgggctt tgctgaccgg caattttgag agcgcagttg acctttgttt acacgataac 2040

cgaatggctg atgccatcat attagccatc gcaggtgggc aggagctctt ggctcaaacg 2100

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cagaagaaat actttgcaaa atcccaaagc aagattacca ggcttatcac tgcggtggtg   2160
atgaagaact ggaaggagat tgttgaatcc tgtgatctta aaaattggag agaggcttta   2220
gctgcggtcc tcacttatgc taagccagat gagttctccg ccctgtgtga tctcttggga   2280
gccaggcttg aaagtgaagg agacagcctc ctgcggactc aggcctgcct ctgctatatc   2340
tgtgccggga atgtagagag attagttgct tgttggacca aagctcaaga tggtagcaac   2400
cccttgtctc tccaggatct gattgagaaa gttgtcatcc tccgaaaagc tgtacagctc   2460
acgcaagccc tggacacgaa cactgtcggg gctcttctgg ccgagaagat gagtcagtat   2520
gccaacttgt tggcagccca gggcagcatt gccgcggcct tggcgtttct gcctgacaac   2580
accaaccagc cagatatcgt gcagctgcgt gacagactct gcagagctca aggcaggtct   2640
gtgccggggc aggagtcctc taggagctcc tacgaggggc agccgctgcc caagggcggg   2700
cccgggccgt ttgctggtca cccgcaggtg tccagagttc aaagtcagca atattatccc   2760
caggttagaa ttgcccctac tgtcactacc tggagtgaca gaactcccac tgccctcccc   2820
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ttcataatgc atggcaacgt tgttccaaat agccctgccc cactgccgac gtctccaggc   2940
cacatgcaca gccagccacc accttatccg cagccgcagc cttaccagcc agcccagcag   3000
tattccttgg gaacaggggg gtcagcagtt tatcgacctc aacagcctgt tgctcctcct   3060
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cctcatatgt acacagcaca gccagcctcc tcccctacct ccagctctgc tcctcttccc   3180
cctcccccttt cctctggagc gtccttccag catggcggac caggagctcc accgtcatct   3240
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gggatccagc agcctcttgc tcaaacaggc atgccaccgt ctttctcgaa gcccaacact   3600
gaaggggccc ccggcgctcc cattgggaac accatccagc atgtgcaggc tctgccaaca   3660
gaaaaaatta ccaagaagcc tattccagac gagcacctca ttctaaagac tacgtttgag   3720
gaccttatcc agcgctgcct ctcctcagct acagaccccc aaaccaagag gaagctggac   3780
gacgccagca aacgcctgga gtgtctgtac gacaaactta gggaccagac actttctcca   3840
acaatcatca gcggtctcca cagcattgcg aggagcattg agacgaggaa ctactctgaa   3900
gggttgaccg tccacactca catcgtcagc accagcaact tcagtgagac ctcagccttc   3960
atgccagtcc tcaaggttgt gctcagccag gccagtaagc tgggcgtctg agacagctgc   4020
catgacctcc gactccgcca cttctccaaa gaaagccatg ctaagactgg gaccagcccc   4080
tcattagcat gtttccatgg cacccgccac ccaggagctt tgacatttct gcaggcaaac   4140
tcccctagaa ctgcttgaac cccccctccc accccgcccg tgctttcctt tgttttaatc   4200
ccctcgccca ttatgatttt cccattctgc aaatagtgtc tttcctggat tacacatagt   4260
gtggtttcct gagggattct gacaaaatgt ggtttttaaa acttgggtgt actttttagg   4320
aaaattgcat ctggctgtgt ataaaacaga cctaaaatag atttctttaa tgtcttccct   4380
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accttcttca cgttcatcag attaaagttg taatcccatc tt                    4422


<210> SEQ ID NO 25
<211> LENGTH: 2704
<212> TYPE: DNA
<213> ORGANISM: Rattus sp.

<400> SEQUENCE: 25

gaattccgga atccggcgag gaaatacatg cactcgctga gaatcgccgg cgccaggacg     60

cagcgccaca aggtgtagcg agtgagtggg gtggggcaag aggggaccca ggagtccccc    120

caggctccca gcgcgcctgc tcctgctctt caatcctgcc ctcggggcgg acggagtgac    180

ccccgccccg accatggtag tgttcaatgg ccttcttaag atcaaaatct gcgaggccgt    240

gagcttgaag cccacagcct ggtcgctgcg ccatgcggtg ggaccccggc cccagacgtt    300

ccttctggac ccctacattg cccttaacgt ggacgactcg cgcatcggcc aaacagccac    360

caagcagaag accaacagtc cggcctggca cgatgagttc gtcactgatg tgtgcaatgg    420

gcgcaagatc gagctggctg tctttcacga tgctcctatc ggctacgacg acttcgtggc    480

caactgcacc atccagttcg aggagctgct gcagaatggg agccgtcact tcgaggactg    540

gattgatctg gagccagaag gaaaagtcta cgtgatcatc gatctctcgg gatcatcggg    600

cgaagcccct aaagacaatg aagaacgagt gtttagggag cggatgcggc caaggaagcg    660

ccaaggggct gtcaggcgca gggtccacca ggtcaatggc cacaagttca tggccaccta    720

cttgcggcag cccacctact gctcccactg tagggatttc atctggggtg tcataggaaa    780

acagggatat caatgtcaag tttgtacctg cgtcgtccac aaacgatgcc atgagctcat    840

tattacgaag tgcgctgggc taaagaaaca ggaaacccct gacgaggtgg gctcccaacg    900

cttcagcgtc aacatgcccc acaagttcgg gatccacaac tacaaggtcc ccacgttctg    960

tgaccactgt ggctccctgc tctggggcct cttgcggcag ggcctgcagt gtaaagtctg   1020

caaaatgaat gttcaccgtc gatgcgagac caacgtggct cccaattgtg ggtggacgc   1080

cagaggaatt gccaaggtgc tggccgatct tggcgttact ccagacaaaa tcaccaacag   1140

tggccagaga aggaaaaagc tcgctgctgg tgctgagtcc ccacagccgg cttctggaaa   1200

ctccccatca gaagacgacc gatccaagtc agcgcccacc tcccccttgtg accaggaact   1260

aaaagaactt gaaaacaaca tccggaaggc cttgtcattt gacaaccgag gagaggagca   1320

ccgagcctcg tcgtctactg atggccagct ggcaagccct ggcgagaacg gtgaagtccg   1380

gcaaggccag gccaagcgct tgggcctgga tgagttcaac ttcatcaagg tgttaggcaa   1440

aggcagcttt ggcaaggtca tgctggccga gctcaagggt aaggatgaag tctatgctgt   1500

gaaggtctta aagaaggacg tcatcctgca ggatgacgac gtggactgca cgatgacaga   1560

gaagaggatt ttggctctgg cgcggaaaca cccttatcta acccaactct attgctgctt   1620

ccagaccaag gaccggctct tcttcgtcat ggaatatgta aacggtggag acctcatgtt   1680

ccagattcag cggtcccgaa aattcgatga gcctcgttcc gggttctatg ctgccgaggt   1740

cacatctgct ctcatgtttc tccaccaaca tggagtgatc tacagggatt tgaaactgga   1800

caacatcctt ctagatgcag aaggtcactc caagctggct gactttggga tgtgcaagga   1860

agggattctg aatggcgtga caactaccac cttctgtggg actcctgact acatagctcc   1920

agagatcctg caggagttgg agtacggccc ctcagtggac tggtgggccc tgggcgtgct   1980
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gatgtacgag atgatggccg ggcagccccc ctttgaagct gacaacgagg acgacttgtt 2040

tgaatccatc cttcacgatg acgttctcta ccctgtctgg cttagcaagg aggctgtcag 2100

catcctgaaa gctttcatga ccaagaaccc gcacaagcgc ctgggctgcg tggcagcaca 2160

gaacggggaa gatgccatca agcaacatcc attcttcaag gagattgact gggtactgct 2220

ggagcagaag aaaatgaagc ccccсttcaa gccgagaatt aaaaccaaga gagatgtcaa 2280

taactttgac caagacttta cccgggaaga gccaatactt acacttgtgg atgaagcaat 2340

cgtgaagcag atcaaccagg aagaattcaa aggcttctcc tactttggtg aagacctgat 2400

gccctgagaa actgcttcac atggagttag ctcactgcaa ggagggtgtt gagacaatcc 2460

cgtgttgcag aggctcagaa tgtctcgaac tattcgtcct ccccagagcc ccagtcccac 2520

atctgctctc ttatttattg catcccctca tcccaggccc tgtccttccc caccctccca 2580

gtgaccagaa ggccctcttt ggtccagact caccaagatc acagatttga actgcgtctg 2640

ctctgtgtgc agtgctaggt ctggagtagc cgtccaccca caaccctgaa gcagcccgga 2700

attc 2704

<210> SEQ ID NO 26
<211> LENGTH: 549
<212> TYPE: DNA
<213> ORGANISM: Rattus sp.

<400> SEQUENCE: 26

agaaaagaat attttattga aacagtttct caattaacaa tggaacaaag tacaatttga 60

ttcaaacctg tccaaccagc ctgaactgct aatgaaagaa ctcaaacaca caggggggaa 120

ctgtgtagga cctttaagtc tctctgccaa tgtggcaaaa aaaaaaaaaa aaaaggtgga 180

gaggggtggg ggtggggtag aaaagacaaa acaactgaca tcaggtttgc tttgcccctg 240

cactggggtg gccctacctc ctgctacagg tgcaatactg gaggacaggc actctaggca 300

tggttatagt gggcaagggg ctctccttcc tggcaaagga cactgtcaag ttgaaccctg 360

acccctcttt ctttacccac aaagcttgcc tgagagtgag gtggcatttt tacattcaca 420

ccatgatctc tgtcccacag ggtcttggga aaggggctca cagtagatag cacatttttg 480

gtcagcccag ggggaagaag gaaagagcct gaagggtaga ttttacagga actggggatg 540

accaggatg 549

<210> SEQ ID NO 27
<211> LENGTH: 1647
<212> TYPE: DNA
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 27

cttccgaaaa gtagtccaca ttgagcaggg tggcctggtc aagcctgaga gggatgacac 60

cgagttccag catccatgtt tcctgcgtgg ccaggaacag ctccttgaga atatcaagag 120

gaaagtgacc agcgtgtcca ccctgaagag tgaggacata aaaatacgcc aggacagtgt 180

caccaggctg ttgacagatg tgcagctgat gaaggggaaa caggagtgta tggactccaa 240

gctcctggcc atgaagcacg agaacgaggc cctgtggcgg gaggtggcca gccttcggca 300

gaagcatgcc cagcagcaaa aagttgtcaa caagctcatc caattcctga tctcactggt 360

gcagtcgaac cggatcctgg gggtgaaaag aaagatccct ctgatgttga gtgacagcag 420

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```
ctcagcacac tctgtgccca agtatggtcg acagtactcc ctggagcatg tccatggtcc    480

cggcccatac tcagctccat ctccagccta cagcagctct agcctttact cctctgatgc    540

tgtcaccagc tctgggccca taatctccga tatcactgag ctggctccca ccagcccttt    600

ggcctcccca ggcaggagca tagatgagag gcctctgtcc agcagcaccc tggtccgtgt    660

caaggaagag ccccccagcc cacctcatag ccctcgggta ctggaggcaa gccctgggcg    720

cccagcctcc atggataccc ctttgtcccc aactgccttc attgactcca tccttcgaga    780

gagcgaacct acccctgctg cctcaaacac agcccctatg gacacaaccg gagcccaagc    840

ccctgcacac ccagccccct ccacccctga gaagtgcctc agcgtagcct gcctagacaa    900

gaacgagcta agtgatcacc tggatgccat ggactccaac ctggacaacc tgcagaccat    960

gctgacaagc cacggcttca gtgtggacac cagtgccctg ctggacctgt tcagcccctc   1020

ggtgaccatg cccgacatga gcctgcctga cctggacagc agcctggcca gcattcagga   1080

acttctatct ccccaagagc ctcccaggcc tattgaggca gagaatagta acgccgactc   1140

aggaaagcag ctggtgcact acacggctca gcctctgttc ctgctggacc ctgatgctgt   1200

ggacacaggg agcagtgaac tgcctgtgct ctttgagctg ggggagagct cctacttctc   1260

tgagggagat gactacacgg atgatcccac catctctctt ctgacgggca ctgaacccca   1320

caaagccaag gaccccactg tctcctagag gtttcagttg tcaggctggc ttgggcctgg   1380

cccccagcct attcctagga cacggctggt cctggggaga caagacagtt gggtagtcca   1440

gggaatccta ggtcaagcca gcacaacccc agtggagcac agatgggact tgggcttggg   1500

cagtaccttg gatcaagagg aagatcctga aggctgcata cctgctgcct tcaccccagc   1560

cccaagtctg ctctctggtc agagcttcac agccacactt ggactgaccc tgcaggttgt   1620

tcataaaatt gtattttgat ttttaat                                       1647
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<210> SEQ ID NO 28
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of artificial sequence: Primer

<400> SEQUENCE: 28

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atatgatcgc ctgcttattc a                                               21
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<210> SEQ ID NO 29
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of artificial sequence: Primer

<400> SEQUENCE: 29

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aaaggtaggc aacattttca c                                               21
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<210> SEQ ID NO 30
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of artificial sequence: Primer

<400> SEQUENCE: 30

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ttgaccacat cgccgaatgc 20

<210> SEQ ID NO 31
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of artificial sequence: Primer

<400> SEQUENCE: 31

agtgcccaca atgagaccaa tc 22

<210> SEQ ID NO 32
<211> LENGTH: 17
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of artificial sequence: Primer

<400> SEQUENCE: 32

cccgtccttg tctccag 17

<210> SEQ ID NO 33
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of artificial sequence: Primer

<400> SEQUENCE: 33

agaaaaagag tgcggatgat g 21

<210> SEQ ID NO 34
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of artificial sequence: Primer

<400> SEQUENCE: 34

agcattttcc agaagagtgg tgtc 24

<210> SEQ ID NO 35
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of artificial sequence: Primer

<400> SEQUENCE: 35

acaaagacgc tgatggctgc 20

<210> SEQ ID NO 36
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of artificial sequence: Primer

<400> SEQUENCE: 36

gagcaccaaa ccacttcctc 20

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<210> SEQ ID NO 37
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of artificial sequence: Primer

<400> SEQUENCE: 37

ctacaacata agggggtctc 20

<210> SEQ ID NO 38
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of artificial sequence: Primer

<400> SEQUENCE: 38

aaatccactt caacctaccg ctc 23

<210> SEQ ID NO 39
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of artificial sequence: Primer

<400> SEQUENCE: 39

tcgtctacaa cataaggggg tctc 24

<210> SEQ ID NO 40
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of artificial sequence: Primer

<400> SEQUENCE: 40

tctatgacag cgagtatgat a 21

<210> SEQ ID NO 41
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of artificial sequence: Primer

<400> SEQUENCE: 41

caagggcact gtggaccaga t 21

<210> SEQ ID NO 42
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of artificial sequence: Primer

<400> SEQUENCE: 42

taaccaccgc atctcttccc tg 22

<210> SEQ ID NO 43
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence

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<220> FEATURE:
<223> OTHER INFORMATION: Description of artificial sequence: Primer

<400> SEQUENCE: 43

tgtgccaagg atttcaaact gg 22

<210> SEQ ID NO 44
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of artificial sequence: Primer

<400> SEQUENCE: 44

cagaatggga gccgtcactt c 21

<210> SEQ ID NO 45
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of artificial sequence: Primer

<400> SEQUENCE: 45

agcgcacttc gtaataatga g 21

<210> SEQ ID NO 46
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of artificial sequence: Primer

<400> SEQUENCE: 46

tttgacaacc gaggagagga gc 22

<210> SEQ ID NO 47
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of artificial sequence: Primer

<400> SEQUENCE: 47

ccttggtctg gaagcagcaa tag 23

<210> SEQ ID NO 48
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of artificial sequence: Primer

<400> SEQUENCE: 48

ggggaactgt gtaggacctt 20

<210> SEQ ID NO 49
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of artificial sequence: Primer

<400> SEQUENCE: 49

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atgtaaaaat gccacctcac t 21

<210> SEQ ID NO 50
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of artificial sequence: Primer

<400> SEQUENCE: 50

ttcaaacctg tccaaccagc c 21

<210> SEQ ID NO 51
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of artificial sequence: Primer

<400> SEQUENCE: 51

ttgtgggtaa agaaagaggg gtc 23

<210> SEQ ID NO 52
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of artificial sequence: Primer

<400> SEQUENCE: 52

tcgcatccgt gtctactcca tc 22

<210> SEQ ID NO 53
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of artificial sequence: Primer

<400> SEQUENCE: 53

ggaagtcctt ttctgtcacc acc 23

<210> SEQ ID NO 54
<211> LENGTH: 21
<212> TYPE: DNA
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<220> FEATURE:
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<400> SEQUENCE: 98

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<210> SEQ ID NO 110
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What is claimed is:

1. A method for determining the effect of a candidate compound on hot-flash symptoms, comprising:

a) contacting a first cell that expresses an estrogen receptor or estrogen related receptor with said candidate compound; and

b) determining the effect of said candidate compound on said first cell's expression of a panel of genes associated with hot flash symptoms.

2. The method of claim 1, wherein said method further comprises comparing said first cell's expression of said panel of genes associated with hot flash symptoms with a reference expression profile of said panel of genes associated with hot flash symptoms.

3. The method of claim 2, wherein said reference expression profile of said panel of genes is the expression profile of the panel of genes following contacting the cell with a compound selected from the group consisting of estradiol, tibolone, raloxifene, and tamoxifen.

4. The method of claim 1, wherein said method further comprises comparing said first cell's expression of said panel of genes associated with hot flash symptoms with a second cell's expression of said panel of genes associated with hot flash symptoms following contact with a compound that has a known effect on hot flash symptoms.

5. The method of claim 4, wherein said compound that has a known effect on hot flash symptoms is selected from the group that consists of estradiol, tibolone, raloxifene, and tamoxifen.

6. The method of claim 2 or **4**, wherein said method comprises determining that said compound decreases the incidence of hot flash symptoms.

7. The method of claim 1, wherein said estrogen receptor is estrogen receptor α.

8. The method of claim 1, wherein said estrogen receptor is estrogen receptor β.

9. The method of claim 1, wherein said cell expresses both estrogen receptor α and estrogen receptor β.

10. The method of claim 1, wherein said cell that expresses the estrogen receptor is selected from the group consisting of a pituitary cell and a hypothalamus cell.

11. The method of claim 10, wherein said cell that expresses the estrogen receptor is selected from the group consisting of a GH3 cell, a GH4 cell, a PR1 cell, a MtT/E-2 cell, an alphaT3-1 cell, a D12 cell, an RCF-8 cell, and a GT1-7 cell.

12. The method of claim 1, wherein said cell's expression of said panel of genes associated with hot flash symptoms is quantified by determining the presence and amount of mRNA expressed from said panel of genes.

13. The method of claim 12 wherein said cell's expression of said panel of genes associated with hot flash symptoms is quantified by a technique selected from the group of reverse transcription real time PCR, quantitative reverse transcription PCR, Northern blot assays, dot blot assays, reverse dot blot assays, RNAse protection assays, 5'-nuclease assays, reporter gene assays, branched DNA assays, bead array assays, and multiplexed array mRNA assays.

14. The method of claim 13, wherein said cell's expression of said panel of genes associated with hot flash symptoms is quantified by a multiplexed array mRNA assay.

15. The method of claim 1, wherein said cell's expression of said panel of genes associated with hot flash symptoms is quantified by determining the presence and amount of protein expressed from said panel of genes.

16. The method of claim 15, wherein said cell's expression of said panel of genes associated with hot flash symptoms is quantified by a technique selected from the group of a western blot assay, an ELISA assay, a cytokine bead array, multiplexed protein detection assays, and an immunofluorescence assay.

17. The method of claim 1, wherein at least one member of said panel of genes is selected from the group consisting of Activin Beta E, Type II Hexokinase, Multi Drug Resistance Gene, Parvalbumin, BAD2, Prolactin, Argininosuccinate Synthetase, Ribonucleoside Reductase 1, Interleukin-18, ARL gene 4, Calpain, EST196325, CPP32, EST208064, 2-alpha-1 globin, Amiloride Binding Protein, Annexin 1, N27, HBP1, D-binding protein, FE65, Protein Kinase C type I, Glutamate Receptor subunit d1, VAP1, Protein Kinase C subspecies epsilon, EST203549, and Heat Shock Transcription Factor 1.

18. The method of claim 17, wherein at least one member of said panel of genes is selected from the group consisting of Type II Hexokinase, Multi Drug Resistance Gene, Parvalbumin, BAD2, Interleukin-18, Calpain, EST196325, Annexin 1, N27, HBP1, and Protein Kinase C subspecies epsilon, and wherein expression of at least one member of said panel of genes is upregulated in said cell following said contact with said candidate compound.

19. The method of claim 17, wherein at least one member of said panel of genes is selected from the group consisting of Type II Hexokinase, Multi Drug Resistance Gene, Parvalbumin, BAD2, Interleukin-18, Calpain, EST196325, Annexin 1, N27, HBP1, and Protein Kinase C subspecies epsilon, and wherein expression of at least one member of said panel of genes is not upregulated in said cell following said contact with said candidate compound.

20. The method of claim 17, wherein at least one member of said panel of genes is selected from the group consisting of Type II Hexokinase, Multi Drug Resistance Gene, Parvalbumin, BAD2, Prolactin, Interleukin-18, Calpain, EST196325, Annexin 1, N27, HBP1, and Protein Kinase C subspecies epsilon, and wherein expression of at least one member of said panel of genes is downregulated in said cell following said contact with said candidate compound.

21. The method of claim 17, wherein at least one member of said panel of genes is selected from the group consisting of Type II Hexokinase, Multi Drug Resistance Gene, Parvalbumin, BAD2, Prolactin, Interleukin-18, Calpain, EST196325, Annexin 1, N27, HBP1, and Protein Kinase C subspecies epsilon, and wherein expression of at least one member of said panel of genes is not downregulated in said cell following said contact with said candidate compound.

22. The method of claim 6, wherein said panel of genes comprises Type II Hexokinase, Multi Drug Resistance Gene, Parvalbumin, BAD2, Prolactin, Interleukin-18, Calpain, EST196325, Annexin 1, N27, and HBP1; wherein expression of Type II Hexokinase, Multi Drug Resistance Gene, Parvalbumin, BAD2, Prolactin, Interleukin-18, Calpain, and EST196325 is upregulated in said cell following said contact with said candidate compound; wherein expression of Annexin 1, N27, and HBP1 is not upregulated in said cell following said contact with said candidate compound; and wherein expression of Protein Kinase C subspecies epsilon is not downregulated in said cell following said contact with said candidate compound.

23. A method for rapidly determining the effects of a plurality of compounds on hot-flash symptoms, comprising:

a) separately contacting a sample of cells that express an estrogen receptor or estrogen related receptor with each member of said plurality of compounds; and

b) assessing the effect of each member of said plurality of compounds on each of said samples of cells' expression of a panel of genes associated with hot flash symptoms, thereby predicting the effect of each of said compounds on hot-flash symptoms.

24. The method of claim 23, wherein said method further comprises comparing said expression of said panel of genes associated with hot flash symptoms by said samples of cells with a reference expression profile of said panel of genes associated with hot flash symptoms.

25. The method of claim 24, wherein said reference expression profile of said panel of genes is the expression profile of the panel of genes following contacting the cell

with a compound selected from the group consisting of estradiol, tibolone, raloxifene, and tamoxifen.

26. The method of claim 23, wherein said method further comprises comparing said expression of said panel of genes associated with hot flash symptoms by said samples of cells with the expression of said panel of genes associated with hot flash symptoms by a sample of cells following contact with a compound that has a known effect on hot flash symptoms.

27. The method of claim 26, wherein said compound that has a known effect on hot flash symptoms is selected from the group that consists of estradiol, tibolone, raloxifene, and tamoxifen.

28. The method of claim 24 or **26**, wherein said method comprises determining that said compound decreases the incidence of hot flash symptoms.

29. The method of claim 23, wherein said estrogen receptor is estrogen receptor α.

30. The method of claim 23, wherein said estrogen receptor is estrogen receptor β.

31. The method of claim 23, wherein said sample of cells expresses both estrogen receptor α and estrogen receptor β.

32. The method of claim 23, wherein said sample of cells that expresses the estrogen receptor is selected from the group consisting of a pituitary cell and a hypothalamus cell.

33. The method of claim 32, wherein said sample of cells that expresses the estrogen receptor is selected from the group consisting of a GH3 cell, a GH4 cell, a PR1 cell, a MtT/E-2 cell, a alphaT3-1 cell, a D12 cell, an RCF-8 cell, and a GT1-7 cell.

34. The method of claim 23, wherein said expression of said panel of genes associated with hot flash symptoms by said sample of cells is quantified by determining the presence and amount of mRNA expressed from said panel of genes.

35. The method of claim 34, wherein said expression of said panel of genes associated with hot flash symptoms by said sample of cells is quantified by a technique selected from the group of reverse transcription real time PCR, quantitative reverse transcription PCR, Northern blot assays, dot blot assays, reverse dot blot assays, RNAse protection assays, 5'-nuclease assays, reporter gene assays, branched DNA assays, bead array assays, and multiplexed array mRNA assays.

36. The method of claim 35, wherein said expression of said panel of genes associated with hot flash symptoms by said sample of cells is quantified by a multiplexed array mRNA assay.

37. The method of claim 23, wherein said expression of said panel of genes associated with hot flash symptoms by said sample of cells is quantified by determining the presence and amount of protein expressed from said panel of genes.

38. The method of claim 37, wherein said expression of said panel of genes associated with hot flash symptoms by said sample of cells is quantified by a technique selected from the group of a western blot assay, an ELISA assay, a cytokine bead array, multiplexed protein detection assays, and an immunofluorescence assay.

39. The method of claim 23, wherein at least one member of said panel of genes is selected from the group consisting of Activin Beta E, Type II Hexokinase, Multi Drug Resistance Gene, Parvalbumin, BAD2, Prolactin, Argininosuccinate Synthetase, Ribonucleoside Reductase 1, Interleukin-18, ARL gene 4, Calpain, EST196325, CPP32, EST208064, 2-alpha-1 globin, Amiloride Binding Protein, Annexin 1, N27, HBP1, D-binding protein, FE65, Protein Kinase C type I, Glutamate Receptor subunit d1, VAP1, Protein Kinase C subspecies epsilon, EST203549, and Heat Shock Transcription Factor 1.

40. The method of claim 39, wherein at least one member of said panel of genes is selected from the group consisting of Type II Hexokinase, Multi Drug Resistance Gene, Parvalbumin, BAD2, Interleukin-18, Calpain, EST196325, Annexin 1, N27, HBP1, and Protein Kinase C subspecies epsilon, and wherein expression of at least one member of said panel of genes is upregulated in said sample of cells following said contact with said candidate compound.

41. The method of claim 39, wherein at least one member of said panel of genes is selected from the group consisting of Type II Hexokinase, Multi Drug Resistance Gene, Parvalbumin, BAD2, Interleukin-18, Calpain, EST196325, Annexin 1, N27, HBP1, and Protein Kinase C subspecies epsilon, and wherein expression of at least one member of said panel of genes is not upregulated in said sample of cells following said contact with said candidate compound.

42. The method of claim 39, wherein at least one member of said panel of genes is selected from the group consisting of Type II Hexokinase, Multi Drug Resistance Gene, Parvalbumin, BAD2, Prolactin, Interleukin-18, Calpain, EST196325, Annexin 1, N27, HBP1, and Protein Kinase C subspecies epsilon, and wherein expression of at least one member of said panel of genes is downregulated in said sample of cells following said contact with said candidate compound.

43. The method of claim 39, wherein at least one member of said panel of genes is selected from the group consisting of Type II Hexokinase, Multi Drug Resistance Gene, Parvalbumin, BAD2, Prolactin, Interleukin-18, Calpain, EST196325, Annexin 1, N27, HBP1, and Protein Kinase C subspecies epsilon, and wherein expression of at least one member of said panel of genes is not downregulated in said sample of cells following said contact with said candidate compound.

44. The method of claim 28, wherein said panel of genes comprises Type II Hexokinase, Multi Drug Resistance Gene, Parvalbumin, BAD2, Prolactin, Interleukin-18, Calpain, EST196325, Annexin 1, N27, and HBP1; wherein expression of Type II Hexokinase, Multi Drug Resistance Gene, Parvalbumin, BAD2, Prolactin, Interleukin-18, Calpain, and EST196325 is upregulated in said sample of cells following said contact with said candidate compound; wherein expression of Annexin 1, N27, and HBP1 is not upregulated in said sample of cells following said contact with said candidate compound; and wherein expression of Protein Kinase C subspecies epsilon is not downregulated in said sample of cells following said contact with said candidate compound.

45. An array, comprising:

a) a non-porous surface; and

b) a plurality of different oligonucleotides connected with said surface, wherein at least one of said oligonucleotides is specific for a member of a panel of genes associated with hot flash symptoms, and wherein each of said different oligonucleotides is connected with said surface in a different predetermined region of said surface.

46. The array of claim 45, wherein at least one of said oligonucleotides hybridizes under stringent conditions to a member of a panel of genes associated with hot flash symptoms.

47. The array of claim 46, wherein said member of said panel of genes is selected from the group consisting of Activin Beta E, Type II Hexokinase, Multi Drug Resistance Gene, Parvalbumin, BAD2, Prolactin, Argininosuccinate Synthetase, Ribonucleoside Reductase 1, Interleukin-18, ARL gene 4, Calpain, EST196325, CPP32, EST208064, 2-alpha-1 globin, Amiloride Binding Protein, Annexin 1, N27, HBP1, D-binding protein, FE65, Protein Kinase C type I, Glutamate Receptor subunit d1, VAP1, Protein Kinase C subspecies epsilon, EST203549, and Heat Shock Transcription Factor 1.

48. The array of claim 47, wherein said member of said panel of genes is selected from the group consisting of Type II Hexokinase, Multi Drug Resistance Gene, Parvalbumin, BAD2, Prolactin, Interleukin-18, Calpain, EST196325, Annexin 1, N27, HBP1, and Protein Kinase C subspecies epsilon.

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